UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

JOÃO WILLIAM BOSSOLANI

LIME AND PHOSPHOGYPSUM IN LONG-TERM NO-TILL: SOIL QUALITY IMPROVING CROP PHYSIOLOGY AND ^{15}N-FERTILIZER RECOVERY IN THE SOIL-PLANT SYSTEM

Botucatu

2022

JOÃO WILLIAM BOSSOLANI

LIME AND PHOSPHOGYPSUM IN LONG-TERM NO-TILL: SOIL QUALITY IMPROVING CROP PHYSIOLOGY AND ^{15}N-FERTILIZER RECOVERY IN THE SOIL-PLANT SYSTEM

Thesis presented to São Paulo State University, College of Agricultural Sciences, to obtain Doctor of Philosophy degree in Agronomy (Crop Science).

Adivisor: Prof. Dr. Carlos Alexandre Costa Crusciol

Co-advisor: Drª. Nídia Raquel Costa

Botucatu

2022

B745l

Bossolani, João William

Lime and phosphogypsum in long-term no-till: soil quality improving crop physiology and 15N-fertilizer recovery in the soil-plant system / João William Bossolani. -- Botucatu, 2022

251 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu

Orientador: Carlos Alexandre Costa Crusciol

Coorientadora: Nídia Raquel Costa

1. soil acidity. 2. root deepening. 3. soil microbiology. 4. nitrogen cycle. 5. (15NH4)2SO4. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agronômicas, Botucatu. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: LIME AND PHOSPHOGYPSUM IN LONG-TERM NO-TILL: SOIL QUALITY IMPROVING CROP PHYSIOLOGY AND 15N-FERTILIZER RECOVERY IN THE SOIL-PLANT SYSTEM

AUTOR: JOÃO WILLIAM BOSSOLANI
ORIENTADOR: CARLOS ALEXANDRE COSTA CRUSCIOL
COORIENTADORA: NÍDIA RAQUEL COSTA

Aprovado como parte das exigências para obtenção do Título de Doutor em AGRONOMIA (AGRICULTURA), pela Comissão Examinadora:

Prof. Dr. CARLOS ALEXANDRE COSTA CRUSCIOL (Participaçao Presencial)
Producao e Melhoramento Vegetal / Faculdade de Ciencias Agronomicas de Botucatu

Prof. Dr. JULIANO CARLOS CALONEGO (Participaçao Virtual)
Producao Vegetal / Faculdade de Ciencias Agronomicas de Botucatu UNESP

Prof. Dr. EDUARDO FAVERO CAIRES (Participaçao Virtual)
Ciência do Solo e Engenharia Agrícola / Universidade Estadual de Ponta Grossa

Prof. Dr. FERNANDO DINI ANDREOTE (Participaçao Virtual)
Ciência do Solo / Escola Superior de Agricultura Luiz de Queiroz

Prof. Dr. RAFAEL OTTO (Participaçao Virtual)
Ciência do Solo / Escola Superior de Agricultura Luiz de Queiroz

Botucatu, 28 de junho de 2022

Dedico esta tese de doutorado aos meus pais João Nelson e Benedita, aos meus irmãos Bruno (*in memoriam*) e Ellen, e ao meu avô Nelson (*in memoriam*), por teram me demonstrado a verdadeira definição de amor, honestidade, dignidade, sacrifício e dedicação, que ajudaram a me tornar a pessoa que sou hoje. Que cada uma das minhas conquistas seja a realização de seus próprios sonhos.

AGRADECIMENTOS

Ao nosso bom Deus por sempre mostrar o caminho e a verdade, e por nos conceder o privilégio da vida (mesmo que as vezes efêmera).

A minha família: Benedita José Oliveira Bossolani (mãe), João Nelson Bossolani (pai), Bruno Henrique Bossolani (*In memoriam*; irmão), Ellen Rita Bossolani (irmã), Nelson Bossolani (*In memoriam*; avô), por sempre me incentivarem a seguir adiante, rumo aos meus sonhos, e por me confortarem e serem meu alicerce nessa vida. Meu muito obrigado do fundo do meu coração. É tudo por vocês.

À Mariley Fonseca, pelo amor, paciência, incentivo, companheirismo e compreensão, tornando possível a consolidação deste trabalho e da manutenção da minha sanidade mental. Agradeço à Deus por tê-la em minha vida.

Agradeço imensamente ao meu orientador e amigo, Dr. Carlos Alexandre Costa Crusciol. É um exemplo ímpar de ser humano e professor. Cada passo dado durante o doutorado foi guiado pelos seus exemplos e ensinamentos. Obrigado pela confiança, incentivo e paciência durante todo este período de orientação. Manifesto aqui minha eterna gratidão pela grande contribuição em minha vida profissional e pessoal nestes anos de convivência; foi uma grande oportunidade e um privilégio para mim.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001, no período entre 01/03/2018 à 31/07/2018.

Um agradecimento especial à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela concessão da bolsa de estudos (de 01/09/2018 à 30/06/2022) e por todo apoio financeiro (processos: 2018/11063-7 e 2019/12764-1). Obrigado pelo incentivo a ciência.

Agradeço imensamente à Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, em especial aos professores e funcionários do Departamento de Produção Vegetal pela dedicação e atenção com os alunos. São exemplos de pessoas e profissionais.

Gostaria de agradecer ao Instituto de Ecologia da Holanda (NIOO-KNAW), especialmente ao Departamento de Ecologia Microbiana e à Dra. Eiko E. Kuramae. Obrigado pela oportunidade ter sido seu aprendiz, por lapidar meus conhecimentos, e por me tornar um cientista melhor. Ainda, estendo meus agradecimentos ao meu

amigo Marcio Leite; obrigado por todas dicas e ensinamentos, pela paciência, e pela importante amizade construída durante o intercâmbio.

Um agradecimento especial ao meu amigo Luiz G. Moretti, por ter sido meu guia desde 2011. Não poderia ter encontrado pessoa melhor para dividir aprendizados, conquistas, dias bons e ruins e, claro, as “buchas” do dia-a-dia. Obrigado do fundo do meu coração.

Partindo da premissa de que não chegamos a lugar algum sozinhos, gostaria de agradecer à todos meus amigos e colegas de trabalho da UNESP/FCA: José R. Portugal, Vitor A. Rodrigues, Tatiani M. Galeriani, Sirlene L. Oliveira, Gabriel O. Neves, Andressa S. Dalla Côrt, Letusa Momesso, Israel A. Filho, Gabriela F. Siqueira, Ariani Garcia, Rafael Vilela, Leila Bernart, Nídia R. Costa, Murilo de Campos, Rafael Neres, Fernanda M. Souza, Lucas M. Jacomassi, Marcela P. Oliveira, Josiane A. V. Oliveira, Letícia P. Mendonça, Isabô M. Pascoaloto, entre outros que participaram desta jornada, pessoas que certamente levarei em minhas lembranças. Peço a Deus que os abençoe em suas respectivas caminhadas por este mundo. Obrigado por toda a ajuda na condução deste trabalho e por terem tornado meus dias melhores.

Aos integrantes da equipe de estagiários, pela demonstração da importância do trabalho em grupo, evidenciando que a união entre pessoas determinadas pode gerar grandes obras, além de comunhão e amizade.

À banca avaliadora pela disposição e tempo dedicado a esse trabalho.

Aos meus amigos de morada, Thiago B. Batista e Gustavo R. F. Oliveira. Obrigado pelos bons momentos de risada, sempre regados de aprendizado. Vocês são exemplos de como cientistas devem ter amor e zelo por aquilo que fazem.

Aos meus amigos de longa data, que apesar da distância, sempre mantiveram-se presentes: Alexandre Pedrinho, Luis F. Merlotti e Flávia C. Meirelles. Meu muito obrigado por tudo.

“Aqueles que se sentem satisfeitos sentam-se e nada fazem. Os insatisfeitos são os únicos benfeitores do mundo”

Walter S. Landor

ABSTRACT

The present study is part of a long-term experiment, started in 2002, at Fazenda Experimental Lageado, belonging to the College of Agricultural Sciences (UNESP/FCA), in Botucatu (SP). Continuing the experiment, which is likely to be the only one in the State of São Paulo with such a duration (19 years), the present Thesis aimed to understand the effect of long-term surface application of lime and phosphogypsum on the soil chemical and biological attributes, root growth, plant nutrition, carbon e antioxidant metabolism, ^{15}N-fertilizer recovery in the soil-plant system, and yield of maize intercropped with ruzigrass and soybean in succession. The field part of this study has been carried out since October 2016, when lime and/or phosphogypsum were last applied. Therefore, here we tested the following treatments: *i*) control (no soil amendments applied), *ii*) phosphogypsum alone, *iii*) lime alone and, *iv*) lime and phosphogypsum combination. Soybean sowing occurred between November and December of each year, whereas maize intercropped with ruzigrass was sown in March of each year. Our results suggested that the combination of lime and phosphogypsum proved to be effective in improving soil fertility up to 1 m depth, reflecting ideal conditions for root growth of soybean and maize intercropped with ruzigrass. As a result of these changes, crop nutrition was also improved, ensuring high photosynthetic metabolism even when periods of water scarcity occurred. In the combined application of lime and phosphogypsum, antioxidant metabolism based on free radical-consuming enzymes also increased, reducing lipid peroxidation of leaf cells. This cascading effect was reflected in the increase in grain yield of both crops. Deepening the understanding of tropical agricultural systems altered by the application of lime and phosphogypsum, our microbiological studies revealed that the joint application of lime and phosphogypsum altered the soil chemical properties in a no-till intercropped system, increasing the relative abundance of total prokaryotes (archaea and bacteria), modulating genes related to the nitrogen cycle. Finally, our results also revealed that the combined application of these inputs increases the recovery of ^{15}N fertilizer [$(^{15}NH_4)_2SO_4$] by maize and ruzigrass in intercropping, and soybean cultivated in crop rotation, which reduced N losses to the environment. Our study highlighted the importance of using soil amendments in tropical agricultural systems, mainly in exploring the synergistic effect of lime with phosphogypsum, aiming to improve the soil chemical and microbiological quality, the nitrogen fertilizer recovery, the photosynthetic and antioxidant metabolism of plants and, consequently, the grain yield of the crops.

Keywords: soil acidity; root deepening; soil microbiology; nitrogen cycle; $(^{15}NH_4)_2SO_4$.

RESUMO

O presente estudo faz parte de um experimento de longa duração, instalado no ano de 2002, na Fazenda Experimental Lageado, pertencente à Faculdade de Ciências Agronômicas da UNESP, em Botucatu (SP). Dando continuidade ao experimento, que provavelmente deve ser o único do Estado de São Paulo com tamanha duração (19 anos), a presente tese objetivou entender o efeito da aplicação superficial de calcário e gesso à longo prazo sobre os atributos químicos e biológicos do solo, crescimento radicular, nutrição das plantas, metabolismos do carbono e antioxidante, recuperaçao do ^{15}N-fertilizante no sistema solo-planta, e produtividade de milho consorciado com *Urochloa ruziziensis* e a soja em sucessão. A parte de campo deste estudo vem sendo conduzida desde outubro de 2016, momento em que foi aplicada pela última vez o calcário e/ou gesso. Portanto, aqui testamos os seguintes tratamentos: *i*) controle (sem aplicação de corretivos do solo), *ii*) gesso, *iii*) calcário e, *iv*) combinação de calcário e gesso. As semeaduras da soja ocorreram entre novembro e dezembro de cada ano, enquanto que o milho consorciado com *Urochloa ruziziensis* foi semeado no mês de março de cada ano. Nossos resultados sugeriram que a combinação de calcário e gesso foi eficaz em melhorar a fertilidade do solo até 1 m de profundidade, refletindo condições ideais para o crescimento radicular de soja e milho consorciados com *U. ruziziensis*. Como resultado dessas mudanças, a nutrição das culturas também foi melhorada, garantindo alto metabolismo fotossintético mesmo quando ocorreram períodos de escassez hídrica. Na aplicação combinada de calcário e gesso, o metabolismo antioxidante baseado em enzimas consumidoras de radicais livres também aumentou, reduzindo a peroxidação lipídica das células foliares. Esse efeito cascata refletiu-se no aumento da produtividade de grãos de ambas as culturas. Aprofundando a compreensão dos sistemas agrícolas tropicais alterados pela aplicação de calcário e gesso, nossos estudos microbiológicos revelaram que a aplicação conjunta destes insumos alterou as propriedades químicas do solo em um sistema de plantio direto consorciado, aumentando a abundância relativa de procariontes totais (archaea e bactérias), modulando genes relacionados ao ciclo do nitrogênio. Por fim, nossos resultados também revelaram que a aplicação combinada desses insumos aumenta a recuperação do ^{15}N fertilizante [$(^{15}NH_4)_2SO_4$] pelo milho e *U. ruziziensis* cultivados em consórcio, e da soja cultivada sucessão, o que reduziu fortemente as perdas de N para o meio ambiente. Nosso estudo destacou a importância do uso de corretivos do solo em sistemas agrícolas tropicais, principalmente na exploração do efeito sinérgico do calcário com o gesso, visando melhorar a qualidade química e microbiológica do solo, a recuperação de fertilizantes nitrogenados, o metabolismo fotossintético e antioxidante das plantas e, consequentemente, a produtividade de grãos das lavouras.

Palavras-chave: acidez do solo; aprofundamento radicular; microbiologia do solo; ciclo do nitrogênio; $(^{15}NH_4)_2SO_4$.

LISTA DE ILUSTRAÇÕES

Chapter 1 – Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics

Supplementary material

LISTA DE TABELAS

Supplementary material

SUMMARY

GENERAL INTRODUCTION

Soil acidity is one of the most limiting factors for crop development by reducing its productive potential. Approximately 50% of the arable area across the globe is affected by problems related to soil acidity (SUMNER; NOBLE, 2003; VON UEXKÜLL; MUTERT, 1995). Most of these soils are located in tropical and subtropical regions, as they present a high degree of weathering, which promotes acidity problems, reduced cation exchange capacity (CEC) and base saturation (BS), in addition to containing high levels of toxic elements such as exchangeable aluminum (Al^{3+}) (CAIRES *et al.*, 2008; CAIRES *et al.*, 2011; CRUSCIOL *et al.*, 2016; FAGERIA; NASCENTE, 2014; LI *et al.*, 2019). Brazil, one of the largest food producers in the world, has about 58% of its territory naturally acidic, which becomes a major problem especially in low-altitude Cerrado, a Biome with predisposition to dry winters and summers with dry spell periods (CUNNINGHAM, 2020).

Liming is a very important practice for the viability of agricultural production, since this operation can neutralize soil acidity, increasing nutrient availability, in addition to providing calcium (Ca^{2+}) and magnesium (Mg^{2+}), and reducing Al^{3+} toxicity, especially at uppermost soil layers. On the other hand, the no-tillage system (NTS) requires no tilling of the soil, reducing liming efficiency (CAIRES; GARBUIO; BARTH, 2008; SANTOS *et al.*, 2018). In this type of production system, liming is usually carried out on the soil surface, without incorporation, promoting great inquiries in the scientific environment due to low low mobility in the profile (low solubility in water) (SORATTO; CRUCIOL, 2008).

Due to the issues that occurs in surface application of lime, studies with phosphogypsum has started. Phosphogypsum is a by-product of obtaining phosphoric acid, basically composed of calcium sulfate ($CaSO_4.2H_2O$). This input, unlike lime, does not correct soil acidity; however it is an alternative in providing Ca^{2+} at deep layers, in addition to reducing Al^{3+} toxic levels throughout the soil profile (CARMEIS FILHO; CRUSCIOL; CASTILHOS, 2017; CRUSCIOL *et al.*, 2019; SORATTO; CRUSCIOL, 2008). Phosphogypsum solubility is higher compared with lime (~150 times), and thus, moving more quickly through the soil profile (COSTA *et al.*, 2018; TIRITAN *et al.*, 2016). Phosphogypsum, therefore, has been used as a complementary input to lime, enhancing the liming benefits to the soil (BOSSOLANI *et al.*, 2020; CRUSCIOL *et al.*, 2019). On the other hand, there are many formulas for

recommending phosphogypsum for Brazilian soils (CAIRES; GUIMARÃES, 2018; VAN RAIJ *et al.*, 2001; VITTI; LUZ, 2004), requiring further investigation as to the best method for each type of agricultural system and soil.

In addition to the benefits for soil chemical attributes, the application of lime, combined or not with phosphogypsum, can also improve soil physics (soil structure), although this effect is not yet fully understood (CARMEIS FILHO *et al.*, 2017; ROTH; PAVAN, 1991). Regarding microbiological indicators of soil quality, the effect of lime and phosphogypsum management has not yet been elucidated, and contradictory results are often obtained, mainly due to the spatial heterogeneity and complexity of the system (BADALUCCO *et al.*, 1992). These results become more incipient when changes occur only over long-term periods (CUSSER *et al.*, 2020; DUVAL *et al.*, 2013).

Liming can increase soil microbial activity (BOSSOLANI *et al.*, 2020; HOLLAND *et al.*, 2018). As a rule, raising the soil pH, increases the mineralization of soil organic matter, in addition to providing a direct impact on the nitrogen cycle potential of soils (BOSSOLANI *et al.*, 2020; HOLLAND *et al.*, 2018). Studies in tropical regions evaluating the effect of lime and phosphogypsum on the soil microbiological activity and structure are scarce, and further investigations are necessary, given the importance of microbiology in increasing the productivity of soil and crops (BOSSOLANI *et al.*, 2021a). Therefore, understanding the effect of these inputs on soil quality is important to predict changes in soil productive balance and the long-term sustainability, and profitability of agricultural systems (GROVER *et al.*, 2017; INAGAKI *et al.*, 2016), especially in tropical region. Furthermore, soil fertility management with lime and phosphogypsum can be used as an important tool to overcome periods of water scarcity (BOSSOLANI *et al.*, 2021b). Tropical regions presents stronlgy predisposition to dry periods throughout the year CARMEIS FILHO *et al.*, 2017, which drastically reduce crop yield. The construction of a fertile profile ensures increased root growth of crops, accessing a greater amount of soil resources (water and nutrients), which leads crops to overcome periods of drought (BOSSOLANI *et al.*, 2021b)

Therefore, this research aimed to evaluate the effect of surface application of lime and phosphogypsum on the combination of soil chemical with physical and biological properties (functional genes and taxonomical profile), root growth, plant nutrition, carbon and antioxidant metabolism, yield components, and the ^{15}N-fertilizer recovery by crops in the soil-plant system.

CHAPTER 1

IMPROVING SOIL FERTILITY WITH LIME AND PHOSPHOGYPSUM ENHANCES SOYBEAN YIELD AND PHYSIOLOGICAL CHARACTERISTICS[1]

Published in: Bossolani, J. W., Crusciol, C. A. C., Moretti, L. G., Garcia, A., Portugal, J. R., Bernart, L., Vilela, R. G., Caires, E. F., Amado, T. J. C., Calonego, J. C., dos Reis, A. R. (2022). Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics. Agronomy for Sustainable Development, 42(2), 1-19. https://doi.org/10.1007/s13593-022-00765-9

Abstract

In tropical no-till systems, applying lime (L) and phosphogypsum (PG) on the soil surface may be a potential strategy for reducing soil acidification and improving soybean root growth, thereby enhancing plant nutrition and physiological responses and, in turn, crop resistance to dry spells. This study evaluated the impact of long-term (17 years) surface soil amendment on soil fertility and soybean root development, nutrition, gas exchange, carbon and antioxidant enzyme activity and grain yield in a tropical region subject to dry spells. The treatments consisted of the following long-term soil amendments: control (no soil amendment); L alone; PG alone; and L + PG (LPG). Liming, especially when combined with PG, improved soil fertility, as evidenced by increases in pH and P, Ca^{2+} and Mg^{2+} levels throughout the soil profile, but reduced Al^{3+} and micronutrients (Fe, Mn, Cu and Zn). The improvements in soil fertility were associated with increased root development throughout the profile. Long-term application of LPG reduced the negative impacts of dry spells on pigment concentrations, gas exchange, Rubisco and sucrose synthase activities and antioxidant metabolism and increased soybean grain yield. Our results reveal that long-term application of LPG is an important approach for increasing the vertical movement of cationic bases and roots in no-till systems to improve soybean nutrition. Long-term amendment with LPG enhanced both carbon and antioxidant metabolism in soybean plants, resulting in higher soybean grain yield, despite the predisposition of this tropical region to dry spells.

Keywords: *Glycine max* (L.) Merrill; soil amendments; soil acidity; Rubisco; Sucrose Synthase; oxidative stress

[1] Chapter written in accordance with the rules of Agronomy for Sustainable Development.

1.1 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is important both as an oil-protein seed crop for livestock and aquaculture feeds and as a biofuel feedstock (Sugiyama et al., 2014). Soybean is unique among crops in that it can supply protein equal in quality to that of animal sources, making it an excellent protein source for the human diet (Hartman et al. 2011; Sugiyama et al. 2014). Demand for soybean and its derivatives has increased in the last decade, challenging the reliability of supply, stock levels, and competitive pricing (Hartman et al. 2011). The anticipated growth of the global population to approximately 10 billion people by 2050 (Desa 2015) and the effects of climate change (Gupta et al. 2020) have heightened the need for sustainable and highly efficient agricultural practices to guarantee global food security (Rellán-Álvarez et al. 2016).

Among the numerous crop system challenges faced by growers, low soil quality and reduced nutrient availability associated with water restriction (due to unpredictable weather or inappropriate management practices) are the main factors responsible for losses in agricultural production, particularly in tropical regions (Rellán-Álvarez et al. 2016; Costa et al. 2018). The detrimental effects of climate change may potentially be alleviated by adopting appropriate soil management practices, particularly soil chemical correction, which is increasingly necessary to improve the acquisition of soil resources by plants (Lal 2009). Soil amendments such as lime (L), a corrective agent for soil acidity with low solubility (Crusciol et al. 2019), and phosphogypsum (PG), a subsurface conditioning agent with moderate solubility (Zoca and Penn, 2017), can improve soil quality (Bossolani et al. 2020a). In no-tillage systems (NTS), L is applied superficially, which may limit its effectiveness due to its poor solubility, whereas PG has greater mobility in the soil profile but does not correct soil acidity (Zoca and Penn, 2017). Thus, combining L and PG can be an alternative for improving the plant growth environment in tropical soils (Bossolani et al. 2020a).

In addition to reducing soil acidity, soil amendment with L + PG neutralizes elements toxic to plants, such as aluminum (Al^{3+}) and manganese (Mn). Aluminum inhibits root growth, resulting in reduced water and nutrient uptake as well as plant growth (Reis et al. 2018), and thus reducing Al^{3+} toxicity promotes appropriate conditions for crop root development, particularly in deep soil layers (Costa et al. 2018). Moreover, amendment with L + PG supplies plants with nutrients such as calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorus (P), and sulfate (SO_4^{2-}-S) (Crusciol et al. 2019;

Bossolani et al. 2020b). Increasing Ca^{2+} availability in the subsurface promotes hormone signaling and root growth (Zoca and Penn, 2017), and root development into deeper soil layers is crucial for avoiding plant injury under water-deficit conditions (Costa et al. 2018). In turn, deeper roots increase soil nutrient availability (*e.g.* Ca^{2+}, Mg^{2+}, P, and SO_4^{2-}-S), resulting in improved plant nutrition and increased photosynthetic activity. Adequate soil nutrient availability promotes photosynthetic pigment formation, energy production, activation of enzymes involved in CO_2 fixation, photoassimilation partitioning for plant development, and the yield index (Gómez et al. 2019). Well-nourished plants are more tolerant of environmental abiotic stresses and have strong antioxidant scavenging systems, which reduces cellular damage caused by reactive oxygen species (ROS) (Santos et al. 2017).

This study focuses on the impact of soil chemical attributes on the physiological and yield responses of soybean to long-term application of soil amendments (Fig. 1). According to Cusser et al. (2020), management recommendations based on short-term studies are generally contradictory and not representative of the system's equilibrium behavior, and therefore long-term studies are necessary to represent the reality of management within an ecosystem. We investigated the impact of L and PG applied alone or in combination on soil fertility and root growth, especially in deeper soil layers. Simultaneously, the effects of these changes on the physiological status of soybean plants were examined under field conditions to understand how photosynthetic/carbon and antioxidant metabolism are altered by the use of soil amendments and converted into crop yield. To adequately represent the real field conditions of soybean cultivated in rainfed areas, we evaluated soybean plants cultivated under tropical field conditions (i.e., without water supplementation and in a region prone to agroclimatic risks with dry spells during the crop cycle) during the second and third growing seasons after the last soil amendment applications (referred to as the first and second growing seasons hereafter).

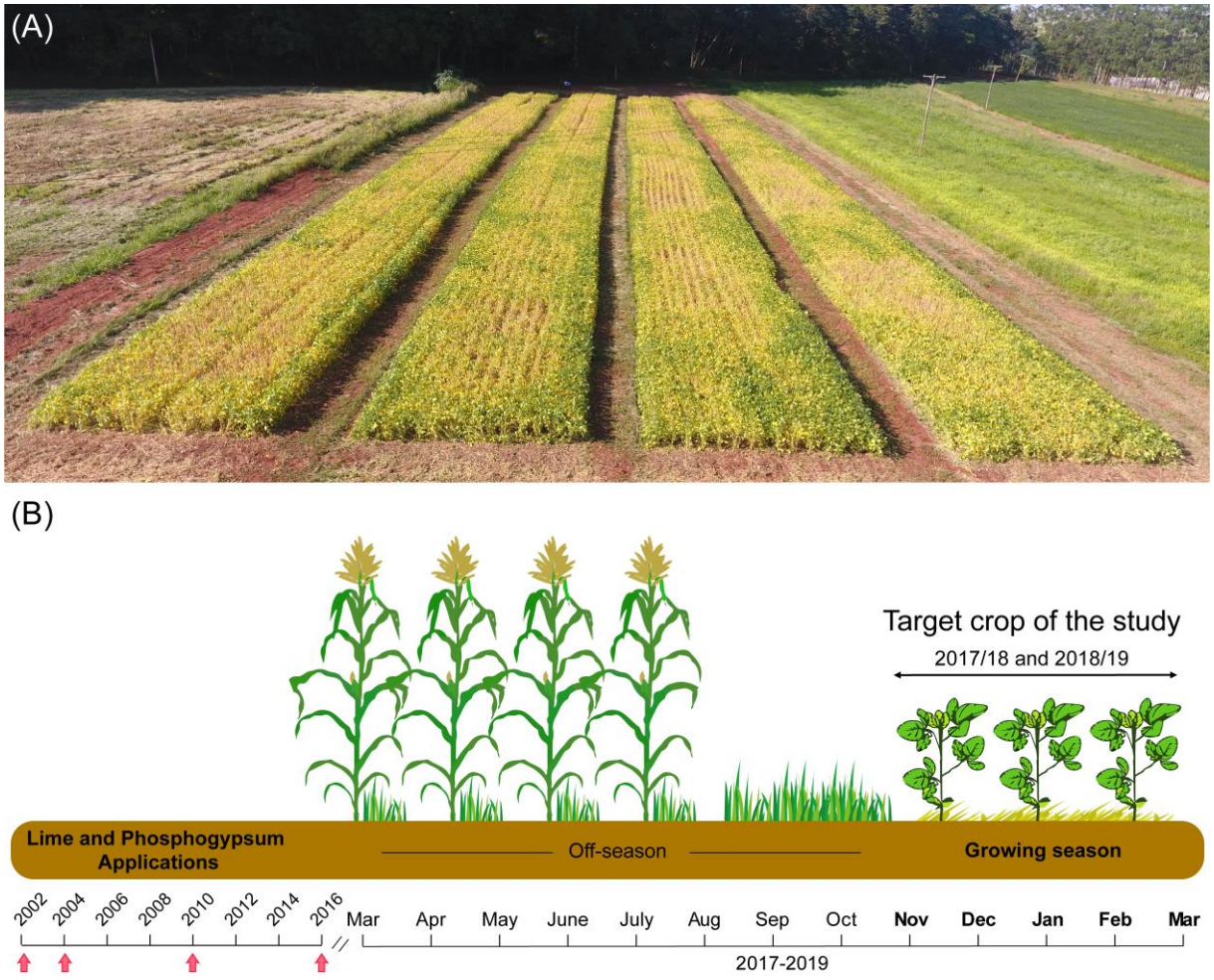


Figure 1. (A) Aerial view of the study area, which is part of a long-term experiment started in 2002. (B) Schematic representation of the cropping system, including the chronological sequences of the soil amendment applications and crops grown during the agricultural year. Red arrows indicate when soil amendment applications occurred. Photographed by J.W. Bossolani.

1. 2 MATERIAL AND METHODS

1.2.1 Site description and experimental design

This study carried out over two growing seasons is part of a long-term field experiment (registered on the GLTEN Metadata Portal; https://www.glten.org/experiments/62) conducted in Botucatu in southeastern São Paulo State, Brazil (22° 83' 3" S, 48° 42' 64" W, elevation 765 m above sea level). The soil was classified as a sandy clay-textured Typic Haplorthox (USDA 2014), which corresponds to Oxisols (Jahn et al. 2006). The chemical and textural properties of the soil at 0.0–0.2 m depth, determined prior to installing the experiment in 2002 (Kiehl

1979; van Raij et al. 2001), were as follows: soil pH ($CaCl_2$), 4.2; soil organic matter (SOM), 21 g kg^{-1}; phosphorus (P_{resin}), 9.2 mg kg^{-1}; potassium (K^+), 1.2 $mmol_c$ kg^{-1}; exchangeable calcium (Ca^{2+}), 14 $mmol_c$ kg^{-1}; exchangeable magnesium (Mg^{2+}), 5 $mmol_c$ kg^{-1}; total acidity at pH 7 (H+Al), 37 $mmol_c$ kg^{-1}; cation exchange capacity (CEC), 57.2 $mmol_c$ kg^{-1}; and base saturation (BS), 35%. The soil particle size distribution (inorganic matrix) in the 0.0–0.2 m layer was 545 g kg^{-1} of clay, 108 g kg^{-1} of silt and 347 g kg^{-1} of sand. The clay content at 0.2–0.4 m was 360 g kg^{-1}. The climate is Cwa, which corresponds to a mesothermic type with dry winters and hot summers according to the Köppen-Geiger climate classification system (Alvares et al. 2013). The long-term (1956–2019) maximum and minimum mean temperatures during the soybean growing season were 26.1 °C and 15.3 °C, and the average annual precipitation was 1,360 mm (Unicamp 2020).

The experiment was designed in randomized complete blocks (RCB) with four replications. The size of each plot was 56.7 m^2 (6.3 m × 9.0 m). The following treatments were applied: (*i*) control (no soil amendment); (*ii*) lime (L); (*iii*) phosphogypsum (PG); and (*iv*) lime + phosphogypsum (LPG). From 2002 to 2019, crop fertilization was performed in the same way in each treatment, and only the soil amendments specified in the treatment were applied. The anhydrous carbonate mineral [$CaMg(CO_3)_2$] containing 166 g kg^{-1} Ca and 105 g kg^{-1} Mg, and the PG contained 200 g kg^{-1} Ca, 150 g kg^{-1} S, <1 g kg^{-1} P, and <1 g kg^{-1} F. At the beginning of the experiment (2002), the L rate was defined according to soil chemical analysis at 0.0–0.2 m depth, and 2.7 Mg ha^{-1} of L was applied with the aim of raising the base saturation (BS) to 70% (van Raij et al. 1997). The PG rate of 2.1 Mg ha^{-1} was calculated according to van Raij et al. (1997) by multiplying the clay content (0.2–0.4 m depth) by a factor 6.

The soil amendments were reapplied when the BS in the L treatment fell to ≤50%, which was verified annually. When L reapplication was necessary, PG was also reapplied (2.1 Mg ha^{-1}). In total, the soil amendments were applied four times, including in 2002, 2004 and 2010 (2.0 Mg ha^{-1} of L and 2.1 Mg ha^{-1} of PG). For the fourth application, which occurred in October 2016, updates to the methods used in Brazil led to changes in the calculation of the L and PG rates. Specifically, the L rate calculation method was revised to consider the 0.0–0.4 m layer. The 0.0–0.4 m layer was chosen as the new criterion based on results obtained by Carmeis Filho et al. (2017) for the same experimental area multiplying by a factor 2 (double of the recommended dose).

Carmeis Filho et al. (2017) found that the classic liming recommendation based on the 0.0–0.2 m layer represents an underestimate for stable cropping systems under no-till with crop rotation and high input of crop residues throughout the agricultural year. Consequently, the rate required to raise the BS to 70% was 13 Mg ha^{-1} of L in 2016. The PG recommendation was also revised following the methodology proposed by Caires and Guimarães (2018). This new recommendation, which was intended to increase Ca^{2+} saturation in the effective cation exchange capacity to 60% in the 0.2–0.4 m soil layer, resulted in a rate of 10 Mg ha^{-1} of PG in 2016. After each of the soil amendment applications in 2002, 2004, 2010, and 2016, a micronutrient-based fertilizer was applied over the total area at rates of 3 kg ha^{-1} B + 1 kg ha^{-1} Cu + 1 kg ha^{-1} Mn + 10 kg ha^{-1} Zn + 0.2 kg ha^{-1} Mo in order to avoid unavailability of micronutrients due to the increase in pH by liming.

The current study reports the results for the soybean crops grown in the second and third years after the last soil amendment reapplication (referred to as the first and second growing seasons hereafter). During the entire period of the experiment (2002–2019), several crops were grown. Details of these previous crops and the applications of L and PG are shown in Table S1. With the exception of the first application of L and PG in 2002, when the no-tillage system was initiated, all soil amendments were surface applied.

1.2.2 Crop sowing and establishment

Soybean (cultivar TMG 7062 IPRO) was sown in November 2017 and 2018 and fertilized with 300 kg ha^{-1} of 04–20–20 ($N–P_2O_5–K_2O$). The seeds were treated with fungicides (carboxin + thiram at 100 g + 100 g a.i. 100 kg^{-1} of seeds) prior to inoculation and sowing. Seed inoculation was performed 1 h before sowing by evenly coating the seeds with an appropriate amount of inoculant containing strains SEMIA 5079 (*Bradyrhizobium japonicum*) and SEMIA 5080 (*Bradyrhizobium diazoefficiens*). Phytosanitary treatments were carried out according to the needs of and recommendations for the soybean crop (Embrapa 2020).

1.2.3 Meteorological data

The tendency for short dry spells in southeastern Brazil during the monsoon season (from October to April) was comprehensively established by Cunningham (2020) based on an analysis of a historical series of 37 austral summer seasons (from 1979 to 2016). During the experimental period, meteorological data (rainfall, solar radiation, wind speed, relative humidity and maximum and minimum temperatures) were measured by an automatic meteorology station installed near the experimental area. The evapotranspiration reference (ET_0) was calculated based on the Penman-Monteith methodology (Allen et al. 1998). Soybean evapotranspiration (ETc) was calculated using the crop coefficient (Kc) for each phenological stage (Allen et al. 1998). Rainfall data were used to monitor the climatological water balance, which was calculated using an electronic spreadsheet (Rolim et al. 1998) following the procedure of Thornthwaite and Mather (1955) to determine the real evapotranspiration (ETr). The climatological water balance in the two soybean growing seasons is shown in Fig. 2.

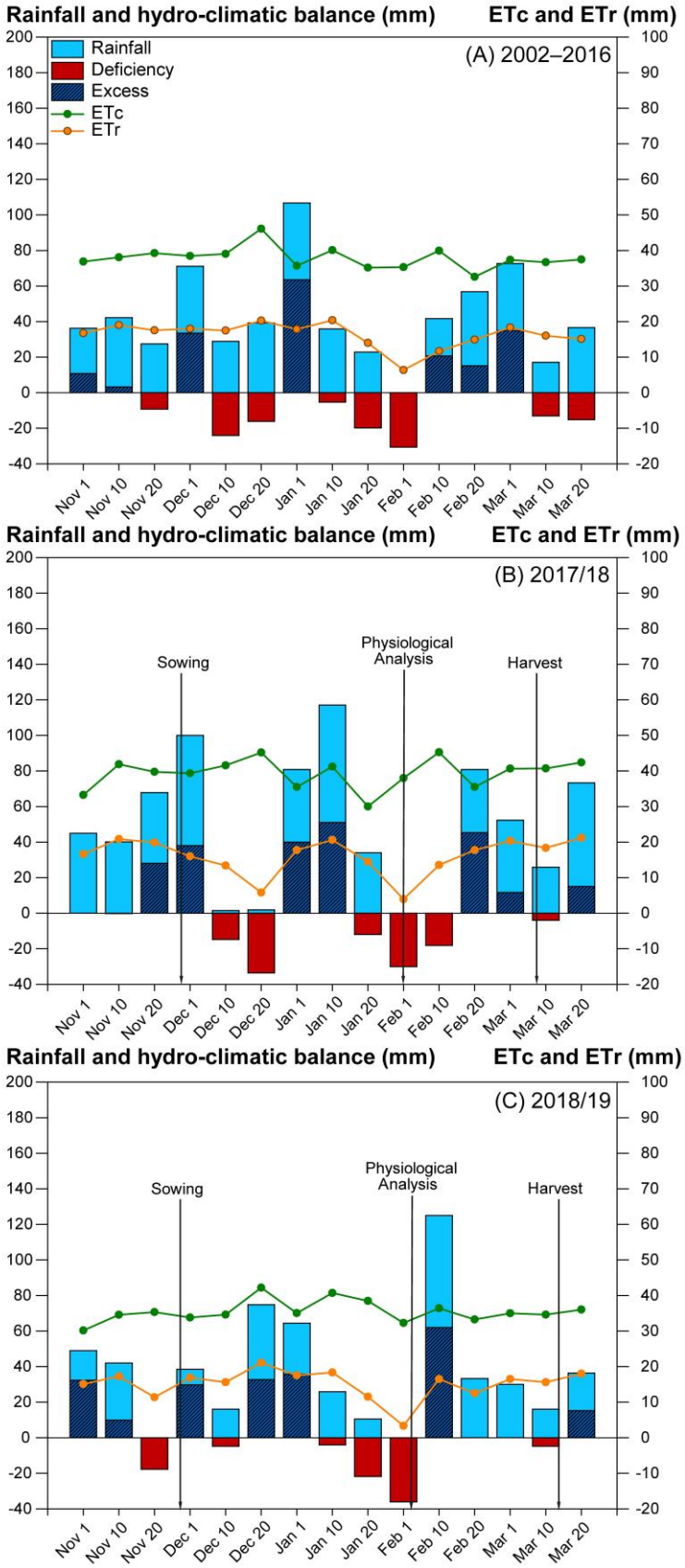


Figure 2. Climatological water balance at Botucatu-SP, Brazil, from (A) 2002 to 2017 and during the soybean crop cycles in (B) 2017/18 and (C) 2018/19. ETc, crop evapotranspiration; ETr, real evapotranspiration.

1.2.4 Soil chemical properties analysis

In October 2018 (24 months after the fourth soil amendment application), eight individual soil samples were randomly collected from each plot at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m using a core sampler with an inner diameter of 50 mm. The samples from a single depth were combined into one sample per depth per plot. The samples were dried, sieved (2 mm) and analyzed according to van Raij et al. (2001). Soil pH was measured using a glass electrode at a soil:0.01 M $CaCl_2$ solution ratio of 1:2.5 (v/v) (van Raij et al., 2001). Soil organic carbon (SOC) was determined by the colorimetric method using a sodium dichromate solution according the method proposed by Heanes (1984). Total acidity (H+Al) was estimated using Shoemaker-McLean-Pratt (SMP) solution (Shoemaker et al. 1961). Exchangeable Al^{3+} was extracted using 1 M KCl solution and quantified by the titrimetric method with 0.025 M NaOH (Bertsch and Bloom 1996). Exchangeable Ca^{2+}, Mg^{2+} and K^{+} were extracted with ion exchange resin (van Raij, et al. 2001) and determined by atomic absorption spectroscopy (AAS). Sulfate (SO_4^{2-}-S) was extracted with 0.01 M calcium phosphate at a 1:2.5 (v/v) soil:solution ratio; the suspension was shaken for 30 min and filtered (Whatman nº 42) before determination of sulfate by a turbidimetric method (Bardsley and Lancaster 1960). Available phosphorus was extracted with ion exchange resin (van Raij et al. 2001) and quantified by a spectrophotometric method. Cationic micronutrients (Fe, Mn, Cu and Zn) were extracted by a solution containing 0.005 M diethylenetriaminepentaacetic acid (DTPA; pH 7.3), 0.1 M triethanolamine (TEA) and 0.01 M $CaCl_2$ and determined by AAS (van Raij et al. 2001). The results were used to calculate the sum of exchangeable bases (SB) as the sum of Ca^{2+}, Mg^{2+}, and K^{+}. Cation exchange capacity was calculated as the sum of (H+Al) + SB. BS was calculated as the ratio of SB to CEC and expressed as a percentage. For the determination of SOM and P, Fe, Mn, Cu, and Zn concentrations, only the soil sampled at a depth of 0.0–0.2 m was analyzed.

1.2.5 Root dry matter

In both growing seasons, at soybean full flowering (R2 phenological stage) (Fehr et al. 1977), eight root subsamples were collected randomly from each plot and combined to form a composite sample. The samples were collected from plant rows

and in the middle of the inter-rows of each sub-plot. A galvanized steel probe with an 82-mm-diameter cutting tip was used at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m. The soybean roots were washed under a flow of swirling water over a 0.5-mm mesh sieve and separated from other plant materials after washing. The root samples were then dried in an oven at 60°C for 72 h to determine root dry matter, which was expressed in g m^{-3} and subsequently estimated as Mg ha^{-1} in the 0.0–1.0 m layer. The root dry matter distribution was calculated by multiplying the ratio of root dry matter in each layer to total root dry matter by 100.

1.2.6 Nutrient concentrations in leaves and grains

Nutrient concentrations were analyzed in diagnostic leaves of soybean at full flowering (R2 soybean phenological stage) (Fehr et al. 1977) and grains after harvest. The diagnostic leaves of soybean were the third fully expanded leaf from the shoot apex. Leaves and grains were dried in an oven at 65°C to constant weight and then ground in a Willey-type mill with a 1-mm screen for macro- and micronutrient analyses. N (leaves and grains) was extracted by sulfuric acid digestion, and the content was determined by the Kjeldahl method. Phosphorus, K, Ca, Mg, S, Fe, Mn, Cu, and Zn (only leaves) were extracted by nitroperchloric acid digestion and determined by AAS as described by AOAC (2016). Crude protein was determined by multiplying the grain N concentration by 6.25 (Krul 2019).

1.2.7 Gas exchange parameters

Gas exchange assessments were performed using diagnostic leaves of fifteen soybean plants per plot at full flowering (R_2 soybean phenological stage) (Fehr et al. 1977) and a Portable Infrared Gas Analyzer CIRAS-3 Portable Photosynthesis System (PP Systems Inc., Amesbury, MA, USA). The readings began after the air temperature in the chamber was adjusted to 28°C with 380 ppm CO_2 and 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) supplied by LED lamps. The minimum equilibration time before performing the reading was 3 min. The measurements were performed between 9:00 and 11:00 am. The net photosynthetic rate expressed as the area (*A*; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}), leaf transpiration (*E*; mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration in the substomatal

chamber (*ic*; mmol CO_2 mol^{-1} air) and water use efficiency (WUE; µmol CO_2 (mmol $H_2O)^{-1}$) were determined.

1.2.8 Physiological analysis of plant leaves

The leaves used for gas exchange analysis were subsequently used for physiological and biochemical analyses. The materials collected were placed in liquid nitrogen and stored at -80°C until biochemical analysis.

1.2.8.1 Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, *b*, total chlorophyll and carotenoids) were determined using five discs cut between the edge and the central vein of the soybean leaves using a paper punch (0.5 cm in diameter). Following Lichtenthaler (1987), the discs were stored for 24 h in 2 mL of N,N-dimethylformamide (DMF) in capped glass vials wrapped in paper-lined aluminum foil, The concentrations of chlorophyll *a*, *b* and carotenoids were quantified using a spectrophotometric method at wavelengths of 664, 647, and 480 nm, respectively.

1.2.8.2 Rubisco (EC 4.1.1.39), and sucrose synthase (Susy, EC 2.4.1.13) activities

Based on the gas exchange data collected in the first growing season, we decided to evaluate the activities of the enzymes Rubisco and Susy in the second growing season to provide support for the previously obtained data.

Total Rubisco activity was measured according to the method described by Reid et al. (1997). Frozen plant material (0.3 g) was extracted with 1.5 mL of buffer containing 58 mM potassium phosphate and 1 mM ethylenediaminetetraacetic acid (EDTA). The material was centrifuged at 14,000 rotations per minute (rpm) for 25 min at 4°C, and the supernatant was maintained at 4°C (Reid et al. 1997). The incubation buffer for Rubisco consisted of 100 mM bicine-NaOH pH 8.0, 25 mM potassium bicarbonate ($KHCO_3$), 20 mM magnesium chloride, 3.5 mM ATP, 5 mM phosphocreatine, 0.25 mM NADH, 80 nkat glyceraldehyde-3-phosphate dehydrogenase, 80 nkat 3-phosphoglyceric phosphokinase and 80 nkat creatine

phosphokinase. A 70-µL aliquot of the supernatant was incubated with 900 µL of the incubation buffer for 5 min at 30°C in the absence of ribulose-1,5-bisphosphate (RuBP) to enable carbamylation of Rubisco. NADP oxidation was initiated by adding 30 µL of 16.66 mM RuBP directly into the cuvette. Readings were carried out using a spectrophotometric method at a wavelength of 340 nm. Rubisco activity was calculated from the difference in the absorbance readings at 0 and 1 min (without removing the cuvette from the spectrophotometer) and expressed in µmol min^{-1} mg $protein^{-1}$.

Susy activity was determined by extracting 0.5 g of frozen plant material with extraction buffer containing 50 mM HEPES buffer pH 7.0, 2 mM $MgCl_2$, 2 mM dithiothreitol (DTT) and 1 mM EDTA according to Dejardin et al. (1997). After centrifugation at 14,000 rpm for 20 min at 4°C, Susy buffer containing 0.1 M MES buffer pH 6.0, 5 mM $MgCl_2$, 0.3 M sucrose and 5 mM uridine 5'-trihydrogen diphosphate (UDP) was add to 0.5 mL of the supernatant in extraction buffer and incubated at 37°C for 30 minutes. The incubation was stopped by adding 100 µL of 30% potassium hydroxide (w/v) and heating at 100°C for 5 min. Readings were carried out using a spectrophotometric method at a wavelength of 540 nm, and the results were expressed in µmol sucrose g^{-1} fresh weight (FW) h^{-1}.

1.2.8.3 Sucrose concentration

The sucrose concentration was determined from 1 g of frozen plant material extracted in 10 mL of MCW solution (60% methanol, 25% chloroform, and 15% water; (v/v/v)). The homogenized material was centrifuged at 8,000 rpm for 10 min at 4°C. An aliquot of 4 mL of the supernatant was removed and mixed with 1 mL chloroform + 1.5 mL distilled water. After the separation phase, the sucrose content was determined in the aqueous phase as described by Bieleski and Turner (1966). To quantify sucrose, 50 µL of extract, 500 mL of 30% KOH, and 2 mL of concentrated H_2SO_4 were added to a glass tube. The mixture was homogenized by vortexing and heated at 100°C for 10 min. Readings were carried out using a spectrophotometric method at a wavelength of 490 nm. The sucrose concentration was determined by reference to a sucrose standard curve and expressed in mg g^{-1} FW.

1.2.8.4 Hydrogen peroxide

The concentration of hydrogen peroxide (H_2O_2) was determined by reaction with potassium iodide (KI) according to Alexieva et al. (2001). Frozen soybean leaves were ground in liquid nitrogen with a mortar and pestle, and 4 mL of 0.1% trichloroacetic acid (TCA) (w/v) was added. The homogenized material was centrifuged at 12,000 rpm for 15 min at 4°C. For the reaction, 200 µL of supernatant, 200 µL of 100 mM potassium phosphate buffer pH 7.5 and 800 µL of KI solution (1 M) were combined and kept on ice for 1 h. After warming to room temperature, readings were carried out using a spectrophotometric method at a wavelength of 390 nm. The leaf concentration of H_2O_2 was calculated by reference to a standard curve and expressed in µmol g^{-1} FW.

1.2.8.5 Lipid peroxidation

Lipid peroxidation was evaluated based on the production of metabolites reactive to 2-thiobarbituric acid (TBA), mainly malondialdehyde (MDA), according to Heath and Packer (1968). The extraction was carried out with 0.8 g of frozen plant material in 4 mL of 0.1% trichloroacetic acid (TCA; w/v) + 20% polyvinylpolypyrrolidone (PVPP; w/v). The homogenized material was centrifuged at 10,000 rpm for 15 min at 4°C, and 250 µL of the supernatant was added to 1 mL of 20% TCA + 0.5% TBA. The samples were heated at 95°C for 30 min and immediately placed on ice for 10 min. The material was centrifuged again for 10 min at 10,000 rpm. Readings were carried out using a spectrophotometric method at wavelengths of 535 and 600 nm, and the results were expressed in nmol MDA g^{-1} FW.

1.2.8.6 Total soluble protein

Leaf samples were homogenized in 100 mM potassium phosphate buffer (pH 7.5) (1 g of tissue to 3 mL of buffer) containing 1 mM EDTA, 3 mM DTT and 0.04 g of PVPP (Azevedo et al. 1998). The resulting homogenate was centrifuged at 10,000 rpm for 30 min at 4°C, and the supernatant was stored at -80°C before determination of antioxidant activity. The total protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard and measured by a

spectrophotometric method at a wavelength of 595 nm. The results and protein extract were used to determine the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR).

1.2.8.7 Superoxide dismutase (SOD, EC:1.15.1.1)

SOD activity was determined according to Giannopolitis and Ries (1977). The reaction was composed of 2 mL of 50 mM potassium phosphate buffer (pH 7.8), 250 µL of 13 mM methionine, 200 µL of 75 mM nitroblue tetrazolium (NBT), 200 µL of 0.1 mM EDTA, 250 µL of 2 µM riboflavin, and 50 µL of protein extract and was incubated in a reaction chamber under illumination by a 15 W fluorescent lightbulb at 25ºC. The tubes were vortexed and placed inside the chamber (total absence of light) for 15 min to allow the formation of the blue formazan compound via the NBT photoreaction. A control for each sample was performed with the same mixture, but these test tubes were covered with aluminum foil to prevent light exposure. After 15 min, the material was vortexed. Readings were carried out using a spectrophotometric method at a wavelength of 560 nm, and the results were expressed in U SOD mg^{-1} protein.

1.2.8.8 Catalase (CAT, 1.11.1.6)

CAT activity was determined according to the method described by Azevedo et al. (1998). The reaction medium was composed of 1 mL of 30 mM H_2O_2 solution in 100 mM potassium phosphate buffer (pH 7.5). The reaction was started by the addition of 25 µL of protein extract. Enzyme activity was determined by the decomposition of H_2O_2 during a 2-min interval using a spectrophotometer at a wavelength of 240 nm. The results were expressed in µmol min^{-1} mg^{-1} protein.

1.2.8.9 Ascorbate peroxidase (APX, EC:1.11.1.11)

APX activity was determined as described by Gratão et al. (2008). The reaction medium was composed of 100 µL of protein extract and 80 mM potassium phosphate buffer solution (pH 7.0) containing 5 mM ascorbate (AsA), 1 mM EDTA and 1.35 mM

H_2O_2. Readings were carried out immediately using a spectrophotometric method at a wavelength of 290 nm for 2 min. The results were expressed in μmol min^{-1} mg^{-1} protein.

1.2.8.10 Glutathione reductase (GR, EC 1.6.4.2)

GR activity was determined as described by Gomes-Junior et al. (2006). The reaction buffer (1.7 mL) was composed of 1 mL of 100 mM potassium phosphate buffer (pH 7.5), 500 μL of 1 mM nitrobenzoic acid (DTNB), 100 μL of 1 mM oxidized glutathione (GSSG) and 100 μL of 0.1 mM NADPH. A 50-μL aliquot of the protein extract was added and vortexed. The solution was then transferred to a cuvette, and the absorbance at 412 nm was immediately recorded for 2 min. The results were expressed in μmol min^{-1} mg^{-1} protein.

1.2.9 Shoot dry matter and grain yield

At soybean flowering (R2 phenological stage), shoots were collected from a 0.25-m^2 area of each plot. The samples were dried in an oven at 60°C for 72 h to determine shoot dry matter. At harvest, grain yield was evaluated based on the relationship between the mass of grains obtained in the interior (5.4 m^2) of each plot and their water content (130 g kg^{-1} of water).

1.2.10 Statistical analysis

All data were first checked for both normality and homoscedasticity. Then, all data were subjected to analysis of variance (F test), and the means of all treatments were compared by the t-test (Fisher's least significant difference (LSD) at $p \leq 0.05$). Results were expressed as the mean ± standard error of the mean. Comparisons of the means of soil chemical properties were conducted for each soil layer to assess treatment effects. Comparisons were not made between different soil depths. Redundancy analysis (RDA) was used to determine the correlations of carbon metabolism and oxidative stress with soil chemical properties. The Monte Carlo permutation test was applied with 999 random permutations to verify the significance of the effects of soil chemical properties on physiological responses. One-way

PERMANOVA (Anderson 2005) was used to group the treatments by similarity. The correlation heatmap was constructed by calculating the Pearson's correlation coefficients ($p \leq 0.05$), and only significant correlations are shown.

1.3 RESULTS

1.3.1 Climatic conditions

Pluvial precipitation from sowing to soybean physiological maturity was 468 and 418 mm in the first and second growing seasons, respectively (Fig. 2). However, the distribution of rain throughout the crop cycle was uneven, with two short dry spells with a negative water balance between November and February in each growing season. In both growing seasons, the first dry spell occurred after the emergence of seedlings, and the second dry spell spanned the beginning of flowering until pod formation (R1–R4 soybean phenological stage). Specifically, in the first growing season, the first dry spell occurred in the final two ten-day periods of December (accumulated rainfall = 3.4 mm; ETc = 43.4 mm; ETr = 19.2 mm; accumulated water deficit (AWD) = -48.3 mm), corresponding to the vegetative period. The second dry spell encompassed the final ten-day period of January and the first two ten-day periods of February (accumulated rainfall = 35 mm; ETc = 37.7 mm; ETr = 21.3 mm; AWD = -60.2 mm), corresponding to full flowering (R2) to pod formation (R4). In the second growing season, the first dry spell occurred in the first ten-day period of December (accumulated rainfall = 16.1 mm; ETc = 34.6 mm; ETr = 31.2 mm; AWD = -4.8 mm), corresponding to the vegetative period. The second dry spell included the final two ten-day periods of January and the first ten-day period of February (accumulated rainfall = 36.4 mm; ETc = 37.2 mm; ETr = 22.2 mm; AWD = -61.9 mm), corresponding to the beginning of flowering (R1) to pod formation (R4).

1.3.2 Soil chemical properties and root development in the soil profile

At 24 months after the final application of L and PG in 2016, all soil fertility parameters differed significantly ($p < 0.01$; Table S2) among the treatments at all depths (Fig. 3). Surface application of L (regardless of PG addition) reduced soil acidity, even in deeper soil layers (Fig. 3A). As expected, application of PG alone did

not change soil pH in any soil layer compared with the control. All soil amendments increased the calcium concentration compared with the control (Fig. 3B), but the effects of L and LPG were superior to those of PG at all depths. Ca^{2+} levels were higher in LPG-amended soil than in L-amended soil up to a depth of 0.6 m and were similar in these two treatments in the 0.6–1.0 m layer. Amendment with L increased Mg^{2+} levels compared with the control regardless of the application of PG, but in the 0.4–0.6 and 0.8–1.0 m layers, LPG provided higher availability of Mg^{2+} compared with L (Fig 3C).

Similar to the effects on Ca^{2+} and Mg^{2+}, L and LPG application increased BS levels at all soil depths compared with the PG and control treatments (Fig. 3D). BS values were slightly higher in the LPG treatment than in the L treatment at all depths except the deepest layer (0.8–1.0 m), whereas BS was similar in the two treatments. BS was higher in the PG treatment than in the control throughout the soil profile, with the exception of the 0.8–1.0 m layer.

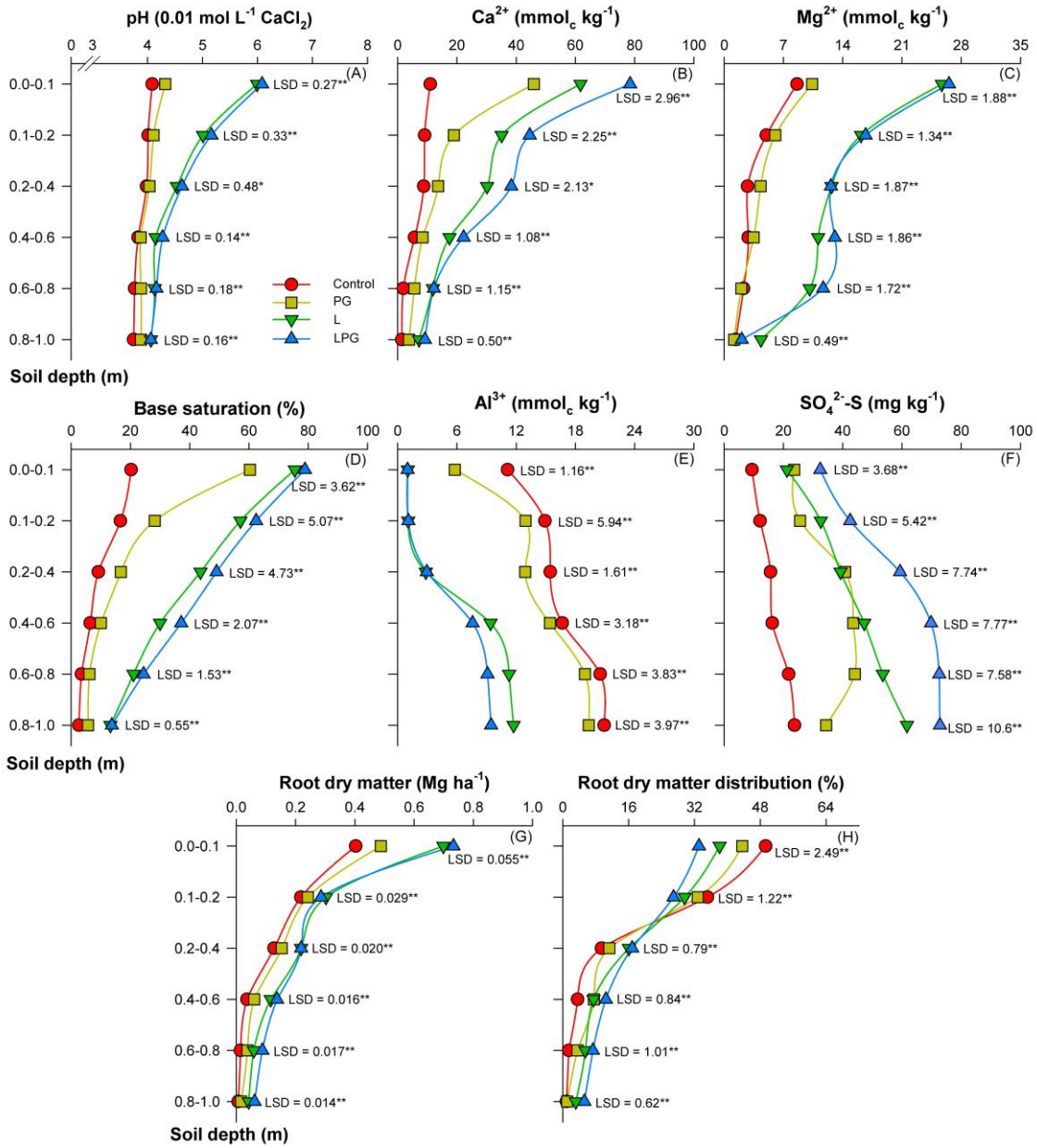


Figure 3. Changes in soil chemical properties [(A) soil pH, (B) Ca^{2+}, (C) Mg^{2+}, (D) base saturation (BS), (E) Al^{3+} and (F) SO_4^{2-}S] and soybean root growth [(G) root dry matter and (H) root dry matter distribution] in the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. * and ** indicate statistical significance at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to the LSD (least significant difference) test. Soil chemical properties and root growth were compared between treatments for each soil depth.

Compared with the control, the concentration of exchangeable Al^{3+} was strongly reduced at all soil depths in the treatments that received L (L and LPG) and up to 0.4 m in the PG treatment (Fig. 3E). SO_4^{2-}-S concentrations were highest at all soil depths in the LPG treatment, followed by the L and PG treatments, in which SO_4^{2-}-S concentrations were similar up to 0.6 m (Fig. 3F). At depths greater than 0.6 m, the

SO_4^{2-}-S concentration was higher in the L treatment than in the PG treatment. SOM and P contents in the 0.0–0.2 m layer were significantly higher ($p < 0.01$; Table S3) in the L and LPG treatments than in the control and PG treatments. By contrast, the concentrations of cationic micronutrients (Fe, Mn, Cu and Zn) were significantly lower ($p < 0.01$; Table S3) in the L and LPG treatments.

There was no significant effect of the soil amendment growing season interaction ($p > 0.05$; Table S4) on soybean root development parameters (Fig. 3G and H). Application of L increased root dry matter production in all layers. In addition, synergetic effects of L and PG on root dry matter production were observed (Fig. 3G) in the uppermost layer (0.0–0.10 m) and at depths greater than 0.6 m. The proportion of roots distributed at a depth of 0.0–0.2 m was highest in the control treatment, followed by the PG treatment (Fig. 3H); consequently, the proportion of roots distributed at depths greater than 0.2 m was lower in these treatments than in the L and LPG treatments. In the L and LPG treatments, the root distribution was more uniform throughout the soil profile, with a higher proportion of roots in deeper layers (0.6–1.0 m depth).

1.3.3 Nutritional status of plants

There was no significant effect of the soil amendment × growing season interaction ($p > 0.05$; Table S6) on the concentration of any nutrient in soybean leaves (Table S5). Compared with the control, application of L (regardless of the application of PG) increased the concentrations of all leaf macronutrients except S, which was higher in the PG treatment. Calcium and Mg concentrations were higher in soybean plants grown in LPG-amended soil than in those grown in L-amended soil. In addition, compared with the LPG treatment, soybean plants in the PG treatment had similar Ca concentrations but lower Mg concentrations. L also increased the K concentration compared with the control, but this increase was slightly smaller when L was combined with PG (LPG). The leaf concentrations of Mn and Zn were lower in the L and LPG treatments than in the PG and control treatments.

1.3.4 Photosynthetic metabolism

The patterns of concentrations of chlorophylls (*a*, *b* and total) and carotenoids were similar (Fig. S1). There was a significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) on the concentrations of all pigments, and the application of L (regardless of PG addition) resulted in the largest increases in pigment concentrations in soybean leaves compared with the control. The concentrations of leaf pigments did not differ between the PG and control treatments and were lower in the second growing season than in the first growing season in these two treatments.

Gas exchange parameters in soybean leaves were positively influenced by the soil amendment growing season interaction ($p < 0.01$; Table S7) (Fig. 4). The application of L, particularly in combination with PG (LPG), impacted leaf gas exchange by increasing *A* (Fig. 4A), *gs* (Fig. 4B) and WUE (Fig. 4E) and reducing *E* (Fig. 4C) and *ic* (Fig. 4D) compared with the PG and control treatments.

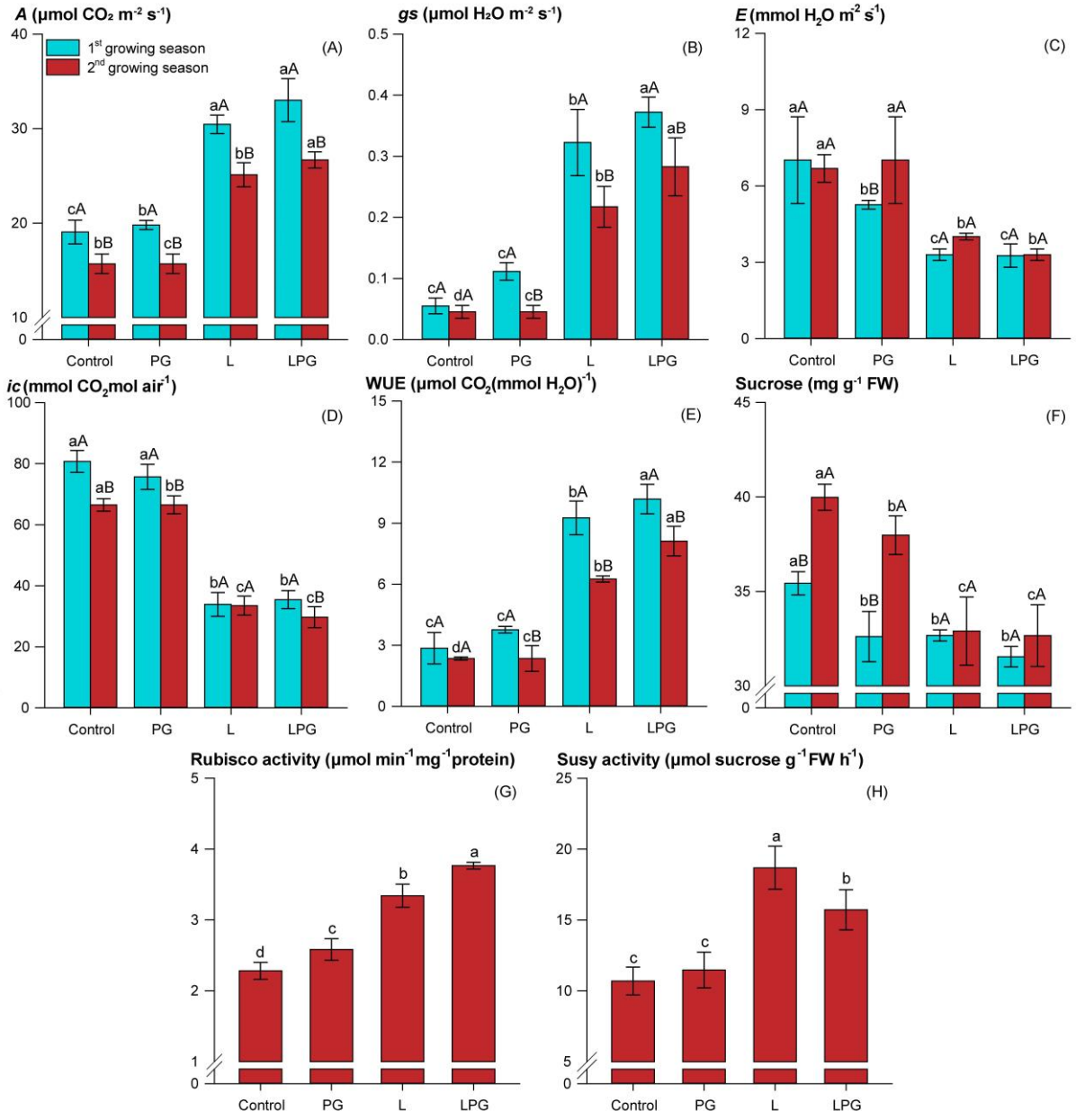


Figure 4. Effects of the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] on (A) net photosynthetic rate (*A*), (B) stomatal conductance (*gs*), (C) transpiration rate (*E*), (D) internal CO_2 concentration (*ic*), (E) water use efficiency (WUE), (F) sucrose concentration, (G) Rubisco activity, and (H) Susy activity in soybean leaves. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

A significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) was observed for leaf sucrose concentrations, which were lower in the L and LPG treatments (Fig. 4F). Additionally, leaf sucrose concentrations were higher in the second growing season than in the first season in the PG and control treatments.

By contrast, Rubisco activity in soybean leaves was highest in the LPG treatment, followed in order by the L, PG, and control treatments (Fig. 4G; Table S7). On the other hand, Susy activity was highest in soybean in the L treatment, followed by the LPG, PG and control treatments (Fig. 4H; Table S7).

1.3.5 Oxidative stress and antioxidant metabolism

The concentrations of H_2O_2 and MDA were significantly influenced ($p < 0.01$; Table S7) by the soil amendments in both growing seasons (Figs. 5A and B). Application of L or LPG reduced oxidative stress (H_2O_2 and MDA production) in soybean plants compared with the PG and control treatments. In general, leaf antioxidant enzyme activities were highest in soybean cultivated in PG-amended or untreated soil (control) (Fig. 5C−F). As oxidative stress increased, the activities of SOD (Fig. 5C), CAT (Fig. 5D), APX (Fig. 5E), and GR (Fig. 5F) increased ($p < 0.01$; Table S7) proportionally. In addition, the activities of these enzymes were higher in the second growing season than the first growing season, particularly for SOD and GR, regardless of the treatment.

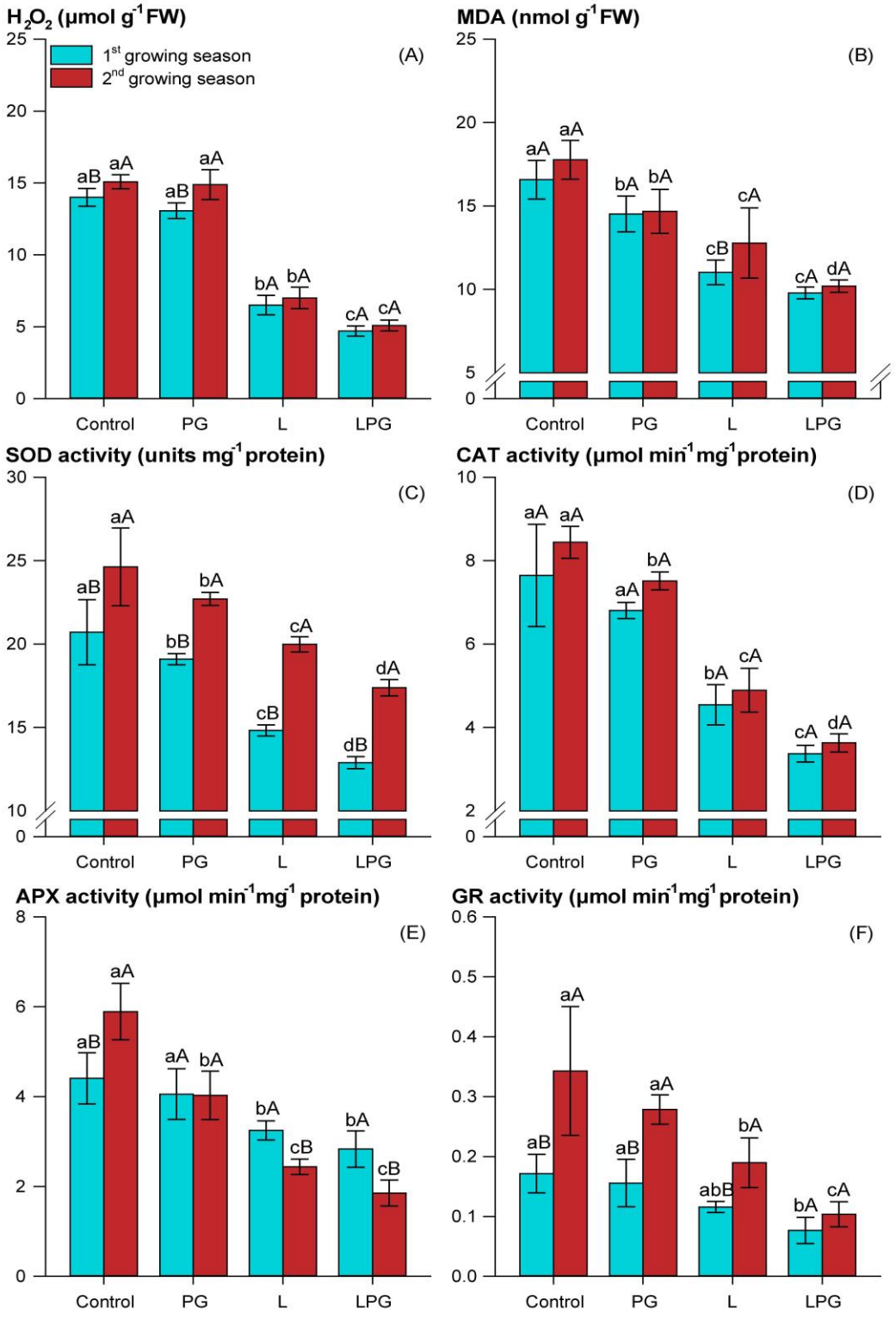


Figure 5. Effects of the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] on (A) hydrogen peroxide (H_2O_2), (B) malondialdehyde (MDA), (C) superoxide dismutase (SOD), (D) catalase (CAT), (E) ascorbate peroxidase (APX), and (F) glutathione reductase (GR)] activity in soybean leaves. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

1.3.6 Soybean shoot dry matter production, grain yield and crude protein in grains

There was a significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) for soybean shoot dry matter production and grain yield. Shoot dry matter and grain yield were higher in the first growing season in all treatments (Fig. 6A). Considering both growing seasons, on average, the treatments that received L (L and LPG) produced ~44% more shoot dry matter than the control and PG treatments. Compared with the control, the PG, L and LPG treatments increased average grain yield over the two growing seasons by ~15, 116 and 140%, respectively (Fig. 6B). The crude protein concentration in grain did not differ between the growing seasons (Fig. 6) and was ~9.5% higher in the treatments that received L (L and LPG) than in the control and PG treatments (Fig. 6C).

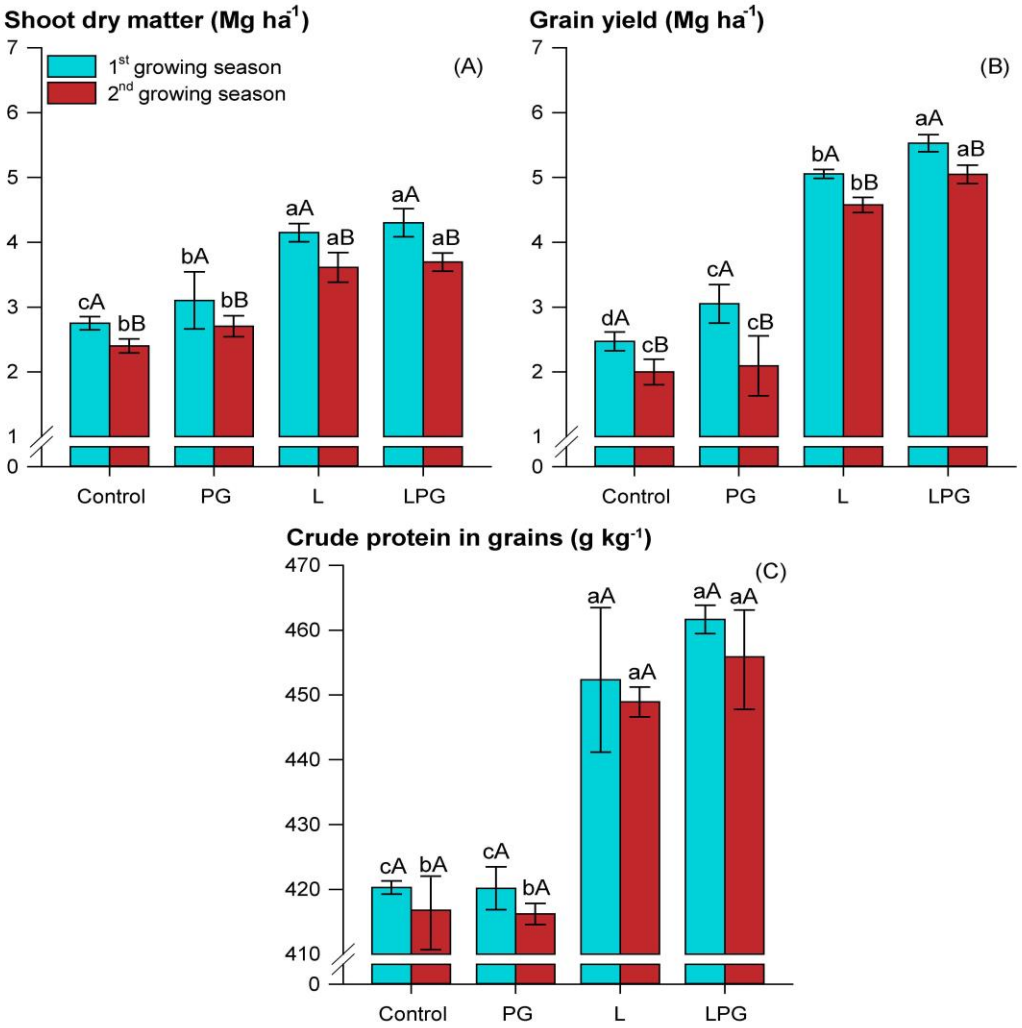


Figure 6. Effects of the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] on (A) shoot dry matter, (B) grain yield and (C)

crude protein in grains of soybean. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

1.3.7 Redundancy analysis and Pearson's correlation analysis of environmental factors (soil chemical properties) and soybean plant parameters

Redundancy analysis (RDA) was performed to identify the main soil factors involved in the responses of carbon fixation metabolism and antioxidant enzymes in soybean plants to the different soil amendment treatments (Fig. 7A). Soil fertility was responsible for 94% of all enzymatic variation. PERMANOVA analysis separated the treatments into three distinct groups ($p < 0.001$), with the control and PG treatments as group 1, the L treatment as group 2, and the LPG treatment as group 3. Monte Carlo permutation analysis indicated that pH ($F = 79.3$; $p < 0.001$), Ca^{2+} ($F = 7.65$; $p = 0.032$), and Mg^{2+} ($F = 3.93$; $p = 0.007$) were the soil factors that most positively influenced enzymes related to carbon metabolism, whereas Al^{3+} ($F = 2.85$; $p = 0.023$) was the soil factor responsible for increasing oxidative stress and antioxidant metabolism in soybean plants.

Correlation analysis among soil and plant parameters demonstrated that improving soil chemical properties (*i.e.* increasing pH, SOM, Ca^{2+}, and Mg^{2+} levels and reducing Al^{3+} levels) improved root development, carbon fixation and its covariates (*i.e.* pigments, gas exchange, Rubisco and Susy activity), reduced oxidative stress, and increased soybean yield (Fig 7B).

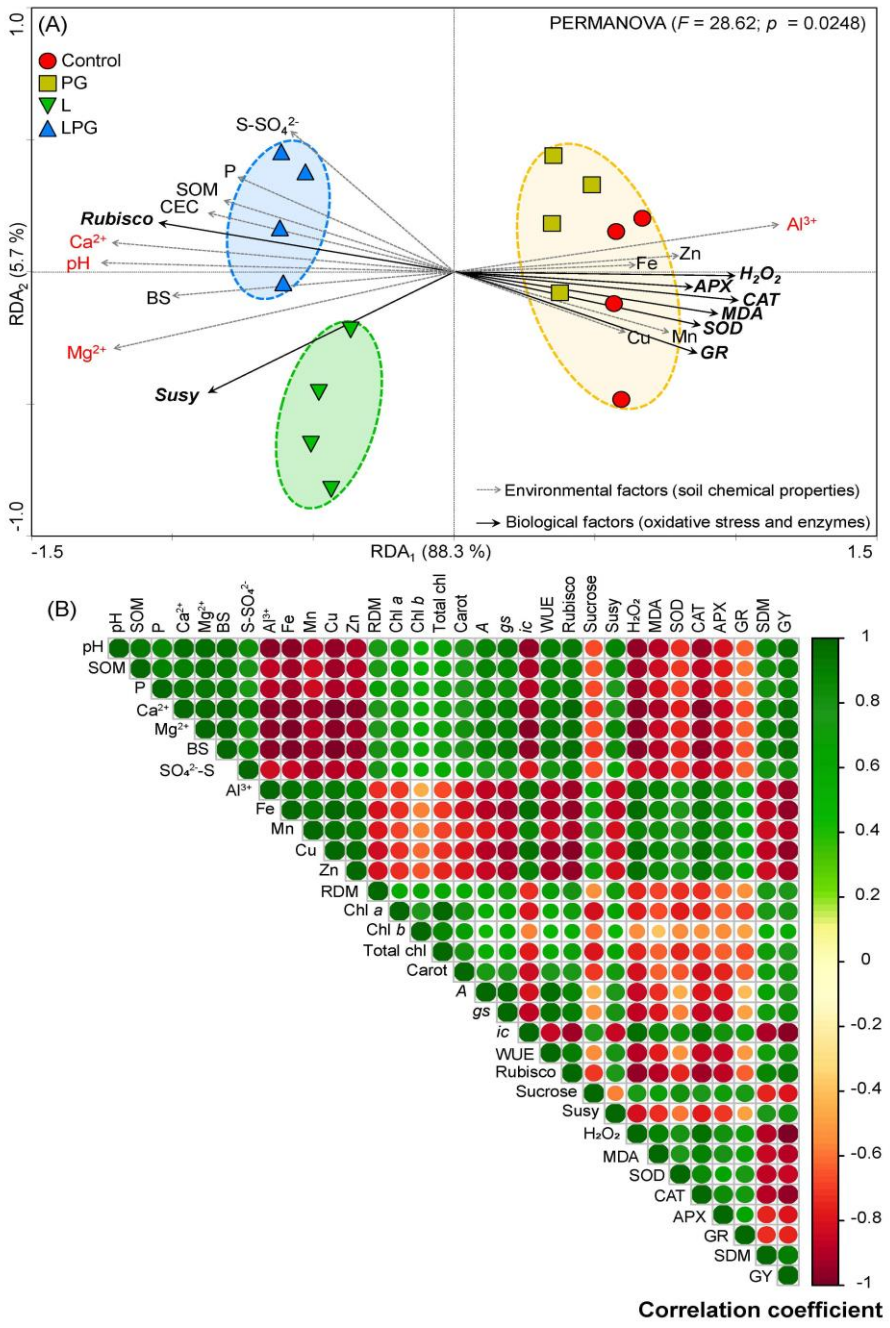


Figure 7. (A) Redundancy analysis (RDA) of the correlations of enzymes related to carbon and antioxidant metabolism with soil chemical properties. The arrows indicate correlations between factors. The canonical axes are labeled with the percentage of total variance explained (%). The significance of correlations was evaluated by a Monte Carlo permutation test, and the significant soil properties are indicated by red color ($p \leq 0.05$). The colored dashed lines indicate significant clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). (B) Heatmap of correlations (Pearson) of soybean physiological, biochemical and agronomic parameters with soil chemical properties. Only significant correlations at $p \leq 0.05$ are shown. Soil organic matter (SOM), base saturation (BS), root dry matter (RDM), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Total chl), carotenoids (Carot), net photosynthetic rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*ic*), water use efficiency (WUE), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), shoot dry

matter (SDM) and grain yield (GY).

1.4 DISCUSSION

1.4.1 Weather conditions

In both growing seasons, rainfall was within the range considered appropriate (450 to 800 mm) to guarantee soybean yield potential according to the recommendations of Farias et al. (2007). However, the water balance was negative in December and January in both seasons, which corresponds to the flowering and initial pod development stages. Short dry periods during the summer cropping season are common in tropical regions (Cunningham 2020). When temperatures and evapotranspiration are high, even short dry periods can cause considerable crop yield losses, especially when such dry periods coincide with critical periods of crop development, such as germination, flowering, and grain filling (Basu et al. 2016). The higher Al^{3+} content in low-fertility acidic tropical soils affects root growth (Bossolani et al. 2021b). Soil amendments can significantly enhance drought tolerance by not only correcting soil acidity and increasing root growth but also improving nutrient availability (Carmeis Filho et al., 2017).

1.4.2 Soil chemical properties, root development and nutritional status of plants

Over the 16-year experimental period, surface-applied L proved to be a viable practice for improving soil chemical properties and alleviating acidity in the soil profile under NTS. Numerous studies have demonstrated that, over the long term, L amendment efficiently increases root growth, improves soil aggregation (Carmeis Filho et al. 2018; Costa et al. 2021; Ferrari Neto et al. 2021), reduces runoff and erosion (Tebebu et al. 2020), and increases soil fertility in the uppermost soil layers (Nora and Amado 2013; Grover et al. 2017; Auler et al. 2019). However, the effects of L on soil chemical properties in subsoil layers have not been comprehensively examined. Overall in the present study, the effects of PG application alone on soil attributes compared with the control were small (with the exception of soil pH, which was unchanged) and were most pronounced in the superficial layers. Although PG alone was not efficient in reducing soil acidity, combining this soil amendment with L (LPG)

potentiated the effects of L (*e.g.*, increased Ca^{2+}, Mg^{2+}, BS, and SO_4^{2-}-S levels and decreased Al^{3+} levels), suggesting that PG is an important complement to L in tropical soils (Crusciol et al. 2019; Bossolani et al. 2020b, 2021a; Moretti et al. 2020). PG is a widely available and inexpensive soil amendment (Zoca and Penn 2017) that provides numerous benefits to agricultural systems, especially when combined with L (Crusciol et al. 2019; Bossolani et al. 2020a, 2021a). Upon application to soil, PG quickly dissociates into SO_4^{2-}-S, which reduces the harmful effects of Al^{3+} throughout the soil profile (Reis et al. 2018), and Ca^{2+} (fertilizing action).

The alleviation of soil acidity by L correlated significantly with increased nutrient availability. In the uppermost layers (0.0−0.2 m), application of L (regardless of PG addition) increased P availability and SOM input despite reducing Fe, Mn, Cu and Zn levels. SOM is the main source of micronutrients for plants, especially in the arable layer (0.0−0.2 m), where SOM content is higher (Fageria and Stone 2008). SOM is also the main source of SO_4^{2-}-S in soils, and the increase in the S concentration in L-amended soil can be explained by the increase in SOM content in the uppermost soil layers due to the higher input of crop residues (shoot and root) over time (Kopittke et al. 2016). The decrease in micronutrient availability due to surface liming can be circumvented by regularly applying micronutrients (as done in this study) or applying conventional fertilizers (N-P_2O_5-K_2O) plus micronutrients in the same input.

The improvements in soil chemical quality were reflected in enhanced soybean root growth and well-nourished soybean plants. The increase in soybean root development is likely attributable to increased availability of exchangeable cations, mainly Ca^{2+}, in deeper layers and reduced Al^{3+} content (Ritchey et al., 1982; Costa et al., 2018). The proportion of roots distributed in deeper layers was highest in the LPG treatment, followed by the L treatment, echoing the pattern of soil fertility parameters. Calcium is required during cell division and elongation (Ritchey et al. 1995), which are disrupted by Al^{3+} (Reis et al. 2018). Therefore, improving chemical attributes throughout the entire soil profile can significantly improve root development and distribution by reducing the concentration of roots in surface layers and encouraging root development in deeper layers of the soil. Longer and deeper roots can efficiently take up water and nutrients from deep layers (Rellán-Álvarez et al. 2016), as evidenced in this study by the significantly higher leaf concentrations of N, Ca, and Mg in soybean plants in the L and LPG treatments. Improving root growth and distribution is fundamental for reducing the negative effects of low water availability during crop

development. Low water availability is common in tropical regions (Carmeis Filho et al. 2017) and was observed during both growing seasons in this study. In a region with constant rainfall and lower evapotranspiration rates (subtropical region), Caires et al. (2008a) observed an increase in the proportion of roots at depths shallower than 0.20 m only. By contrast, root development increased at depths of up to 1.0 m under tropical conditions in the present study, highlighting the importance of amendment with L and PG to guarantee food production under unpredictable weather (Rellán-Álvarez et al. 2016) in a scenario of climate change.

1.4.3 Leaf pigments, gas exchange and carbon metabolism

Leaf pigment concentrations (Chl *a*, *b*, total and carotenoids) were highest in the treatments that received L (L and LPG), probably due to higher soil nutrient availability and nutrient uptake by soybean roots. In photosynthesis, chlorophylls are responsible for capturing light, which excites electrons used to produce reducing and energetic compounds (NADPH and ATP) for reactions of the Calvin cycle (Croft et al. 2017). Carotenoids act as antenna pigments for capturing light and protecting the photosynthetic apparatus against photodestruction by reactive oxygen species (ROS) during abiotic environmental stresses (Gómez et al. 2019).

Pearson correlation analysis indicated a positive relationship between leaf pigment concentrations and the photosynthetic potential of soybean plants. In the L and LPG treatments, the *A* rate increased to the detriment of soybean leaf pigment concentrations. Heat and water stress are exacerbated in tropical environments, resulting in greater declines in crop yields (Paustian et al. 2004). However, under the conditions of the present study, *gs* and WUE increased proportionally with the *A* rate. The *A* rate is mainly reduced through stomatal closure or metabolic impairment (Basu et al. 2016), as observed in the control and PG treatments. In these treatments, *gs* was significantly lower, whereas *ic* (indicating low CO_2 fixation) and *E* were high, suggesting dysfunction of stomatal control. Stomatal control is the immediate plant response to drought stress (Gupta et al. 2020).

In photosynthesis, reducing power and energy produced in the photochemical phase are used to fix CO_2, and this process is highly dependent on tight coordination of enzymatic activity and production of metabolic intermediates (Choudhury and Behera 2001). Metabolic impairment during low water and nutrient availability is mainly

caused by changes in carbon metabolism (Basu et al. 2016). CO_2 fixation efficiency is highly dependent on Rubisco activity, which was higher in the L and, in particular, LPG treatments, corroborating the positive correlations of pigment concentrations with gas exchange. Our findings also indicated changes in source-sink relationships, which determine the grain yield of crops (Ceylan et al. 2016). Low translocation of carbohydrates from source to sink implies less filling of grains or even less capacity to promote root growth, reducing crop establishment under low water availability (Li et al. 2015). Thus, the sink strength (ability of an organ to import photoassimilates) (Basu et al. 2016) is much lower in plants established in low-fertility environments. Furthermore, the low Susy activity in soybean leaves in the control and PG treatments suggests that the process of cleavage of sucrose molecules by Susy (and invertases) for metabolism and conversion into energy and carbon skeletons for plant metabolism was greatly compromised by the poor soil fertility and nutrition (Stein and Granot 2019).

The main soil factors driving soybean carbon metabolism were verified by RDA and confirmed by correlation analysis. PERMANOVA segregated the treatments into three groups: LPG; L; and control and PG. This result indicates that PG application alone was not sufficient to improve soil attributes and, in turn, alter carbon metabolism in soybean plants compared with the control treatment. By contrast, L increased carbon metabolism, and the effects of L were even greater when combined with PG (LPG). Alleviation of soil acidity was the primary factor responsible for increasing Ca^{2+} and Mg^{2+} availability and reducing Al^{3+}, which enhanced Rubisco and Susy activities. Calcium and Mg are essential nutrients for photosynthesis and are involved in many other plant development pathways, such as cell division and elongation and resistance to environmental stress (Wang et al. 2019). Calcium participates in stomatal movements and regulates photosynthetic enzymes during carbon assimilation and chloroplast movement in photosynthetic cells depending on light conditions. Low Mg^{2+} availability decreases photosynthetic capacity by reducing the activities of Rubisco and ATPases (Cakmak and Kirkby 2008), and Mg^{2+} is also an essential component of the chlorophyll molecule and plays a central role in partitioning of photoassimilates by plant tissues (Ceylan et al. 2016). Soybean plants are highly sensitive to Al^{3+} (Reis et al. 2018), as confirmed by the negative impacts of the high concentrations of Al^{3+} in the control and PG treatments on soybean plant parameters. At toxic concentrations, exchangeable Al^{3+} decreases plant root growth and WUE (Carmeis Filho et al. 2017; Reis et al. 2018), compromising crop development and yield.

1.4.4 Antioxidant metabolism

Continuous photosynthetic reactions under low water and nutrient availability result in the accumulation of ROS (Cuypers et al. 2010) such as singlet oxygen ($^1O_2^-$), hydroxyl radicals (OH^-) and H_2O_2 (Farmer and Mueller 2013). ROS can cause serious damage to the photosynthetic apparatus, resulting in lipid peroxidation (Loix et al. 2018). To mitigate the effects of oxidative stress, antioxidant enzymes are activated that catalyze reactions of ROS to reduce cell damage (Farooq et al. 2019). In the L and LPG treatments, the activities of the enzymes SOD, CAT, APX, and GR in soybean plants were low, indicating sufficient mitigation of ROS. On the contrary, the activities of these enzymes and H_2O_2 concentrations were high in the control and PG treatments, demonstrated that the ability of these enzymes to eliminate ROS was inadequate (Farmer and Mueller 2013). In addition, excessive accumulation of carbohydrates in source tissues increases ROS production, especially in chloroplasts, limiting photosynthesis via a negative feedback effect (Cakmak and Kirkby 2008).

RDA and correlation analysis clearly indicated that soils with higher Ca^{2+} and Mg^{2+} availability had improved carbon metabolism and lower oxidative stress due to the roles of these nutrients in photosynthesis. In addition, the resistance of plants to environmental abiotic stress was higher in soils with higher Ca^{2+} and Mg^{2+} availability but reduced in soils with lower availability of these nutrients and higher Al^{3+} levels.

1.4.5 Soybean shoot dry matter production, grain yield and crude protein in grains

The differences in soil fertility, root development, and crop physiological responses among the treatments were reflected in soybean shoot dry matter and grain yield. The results demonstrated that combining L with PG (LPG treatment) is a viable strategy to improve the productive capacity of acidic tropical soils under NTS (Nora et al. 2017; Crusciol et al. 2019; Bossolani et al. 2020a, b). Notably, the effects of the treatments on grain yield were greater than those on shoot dry matter, although the trends were similar. This difference can be explained by the compromised transport of carbohydrates over a long distance (source-sink) (Ceylan et al. 2016), which reduces grain filling. Grain filling is dependent on the translocation of carbohydrates from source-to-sink organs, a process mediated by Mg^{2+} nutrition (Cakmak and Kirkby 2008). In addition, sucrose is the main carbohydrate transported (via phloem) to grains,

and the production of sucrose is strongly influenced by Mg^{2+} nutrition (Ceylan et al. 2016). Our results are consistent with these characteristics of grain filling, as the plants established in soils that received liming (L and LPG) had higher Mg^{2+} nutrition (higher Mg^{2+} availability and uptake by increasing root growth) and less sucrose accumulation in leaves (greater translocation efficiency), culminating in higher grain yield. In addition, the improvements in soybean metabolism and photoassimilates translocation to grains resulted in a significant increase in the concentration of crude protein in soybean grains. Crude protein concentration is an important characteristic related to grain quality and plays a critical role in human and animal nutrition (Karr-Lilienthal et al. 2004).

This study in a tropical region clearly demonstrated that liming benefits soil properties and soybean development. In addition, the combined application of L and PG (LPG) potentiated the beneficial effects of L, providing greater support for grain production, especially in periods of low water availability during soybean development.

1.5 CONCLUSIONS

This study makes an important contribution to understanding the long-term changes below- and aboveground in response to lime and phosphogypsum application. Our results demonstrate that liming is an important tool for improving acidic, low-fertility soils and that the effects of liming are enhanced by phosphogypsum amendment. The combined application of liming and phosphogypsum increased soybean root growth and the ability of plants to acquire water and nutrients from deeper soil layers. These findings have important implications for corrective soil management practices, particularly for the surface application of lime and/or phosphogypsum to increase soybean metabolism. The chemical properties of soil clearly impact soybean metabolism and production, and high-fertility soils enable high photosynthetic performance coupled with increased antioxidant activity. Long-term management with lime and phosphogypsum is therefore an effective approach to increase the grain yield of soybean cultivated in tropical regions predisposed to short dry spells.

Funding

This study was funded by the São Paulo Research Foundation (FAPESP) (Grant 2018/11063-7 and 2019/12764-1) and the National Council for Scientific and

Technological Development (CNPq) (Universal Research Project: 421637/2018-8).

Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable.

Consent to participate

Not applicable**.**

Consent for publication

All authors whose names appear on this submission approved the version to be published and agree to be accountable for all aspects of the work and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Code availability

Not applicable.

Authors' contributions

J.W.B. and C.A.C.C. contributed to the design and execution of this research, data analysis, and writing and formatting the manuscript. L.G.M., A.G., J.R.P., L.B., R.G.V., J.C.C., E.F.C., T.J.C.A., and A.R.R. rewrote, discussed and commented on the manuscript. All authors contributed significantly to this manuscript and approved it for publication.

Acknowledgements

C.A.C.C., E.F.C., T.J.C.A., J.C.C. and A.R.R. would like to thank the National Council for Scientific and Technological Development (CNPq) for an award for

excellence in research. We also thank the Calcário Guapirama Company (www.calcarioguapirama.com.br) for providing the sedimentary lime used in this experiment.

REFERENCES

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344. https://doi.org/https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. Fao, Rome 300:D05109

Alvares CA, Stape JL, Sentelhas PC, et al (2013) Köppen's climate classification map for Brazil. Meteorol Zeitschrift 22:711–728. https://doi.org/https://doi.org/10.1127/0941-2948/2013/0507

Anderson MJ (2005) Permutational multivariate analysis of variance. Dep Stat Univ Auckland, Auckl 26:32–46

AOAC (2016) Official methods of analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5

Assad ED, Sano EE, Masutomo R, et al (1993) Veranicos na região dos cerrados brasileiros frequência e probabilidade de ocorrência. Pesqui. Agropecuária Bras. 28:993–1003

Auler AC, Caires EF, Pires LF, et al (2019) Lime effects in a no-tillage system on Inceptisols in Southern Brazil. Geoderma Reg 16:e00206. https://doi.org/10.1016/j.geodrs.2019.e00206

Azevedo Ra, Alas RM, Smith RJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol Plant 104:280–292. https://doi.org/https://doi.org/10.1034/j.1399-3054.1998.1040217.x.

Bardsley CE, Lancaster JD (1960) Determination of reserve sulfur and soluble sulfates in soils. Soil Sci Soc Am J 24:265–268. https://doi.org/https://doi.org/10.2136/sssaj1960.03615995002400040015x

Basu S, Ramegowda V, Kumar A, Pereira A (2016) Plant adaptation to drought stress. F1000Research 5:1–10. https://doi.org/10.12688/F1000RESEARCH.7678.1

Bertsch PM, Bloom PR (1996) Aluminum. Methods Soil Anal Part 3 Chem Methods 5:517–550

Bieleski RL, Turner NA (1966) Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. Anal Biochem 17:278–293

Bossolani JW, Crusciol CAC, Leite MFA, et al (2021a) Modulation of the soil microbiome by long-term Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. Soil Biol Biochem 156:. https://doi.org/10.1016/j.soilbio.2021.108188

Bossolani JW, Crusciol CAC, Merloti LF, et al (2020a) Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375:114476. https://doi.org/10.1016/j.geoderma.2020.114476

Bossolani JW, Crusciol CAC, Portugal JR, et al (2021b) Long-term liming improves soil fertility and soybean root growth, reflecting improvements in leaf gas exchange and grain yield. Eur J Agron 128:126308. https://doi.org/10.1016/j.eja.2021.126308

Bossolani JW, Santos FL, Meneghette HHA, et al (2020b) Soybean in Crop Rotation with Maize and Palisade Grass Intercropping Enhances the Long-term Effects of Surface Liming in No-till System. J Soil Sci Plant Nutr 20:1–12. https://doi.org/10.1007/s42729-020-00347-2

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/https://doi.org/10.1006/abio.1976.9999.

Caires EF, Corrêa JCL, Churka S, et al (2006) Surface application of lime ameliorates subsoil acidity and improves root growth and yield of wheat in an acid soil under no-till system. Sci Agric 63:502–509. https://doi.org/10.1590/S0103-90162006000500013

Caires EF, Garbuio FJ, Churka S, et al (2008a) Effects of soil acidity amelioration by surface liming on no-till corn, soybean, and wheat root growth and yield. Eur J Agron 28:57–64. https://doi.org/10.1016/j.eja.2007.05.002

Caires EF, Guimarães AM (2018) A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. Agron J 110:1987–1995. https://doi.org/10.2134/agronj2017.11.0642

Caires EF, Pereira Filho PRS, Zardo Filho R, Feldhaus IC (2008b) Soil acidity and aluminium toxicity as affected by surface liming and cover oat residues under a no-till system. Soil Use Manag 24:302–309. https://doi.org/10.1111/j.1475-2743.2008.00166.x

Cakmak I, Kirkby EA (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol Plant 133:692–704. https://doi.org/10.1111/j.1399-3054.2007.01042.x

Carmeis Filho ACA, Crusciol CAC, Castilhos AM (2017) Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. J Agric Sci 155:1093–1112. https://doi.org/10.1017/S0021859617000235

Carmeis Filho ACA, Crusciol CAC, Guimarães TM, et al (2018) Changes in Soil Physical Properties and Carbon Protection Mechanisms by Surface Application of Lime in a Tropical No-Tillage System. Soil Sci Soc Am J 82:56–65. https://doi.org/10.2136/sssaj2017.04.0120

Ceylan Y, Kutman UB, Mengutay M, Cakmak I (2016) Magnesium applications to growth medium and foliage affect the starch distribution, increase the grain size and improve the seed germination in wheat. Plant Soil 406:145–156. https://doi.org/10.1007/s11104-016-2871-8

Choudhury N. ., Behera RK (2001) Photoinhibition of photosynthesis: Role of carotenoids in photoprotection of chloroplast constituents. Photosynthetica 39:481–488. https://doi.org/https://doi.org/10.1023/A:1015647708360

Costa CHM, Carmeis Filho ACA, Crusciol CAC, et al (2018) Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. Crop Pasture Sci 69:488–505. https://doi.org/10.1071/CP17233

Costa CHM, Wander MM, Crusciol CAC, et al (2021) Long-term effects of lime and phosphogypsum on soil carbon and nitrogen and physical attributes under tropical no-till. Soil Sci Soc Am J 85:328–339. https://doi.org/10.1002/saj2.20182

Croft H, Chen JM, Luo X, et al (2017) Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. Glob Chang Biol 23:3513–3524. https://doi.org/10.1111/gcb.13599

Crusciol CAC, Marques RR, Carmeis Filho ACA, et al (2019) Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr Cycl Agroecosystems 115:347–372. https://doi.org/10.1007/s10705-019-10017-0

Cunningham C (2020) Characterization of dry spells in Southeastern Brazil during the monsoon season. Int J Climatol 40:4609–4621. https://doi.org/10.1002/joc.6478

Cusser S, Bahlai C, Swinton SM, et al (2020) Long-term research avoids spurious and misleading trends in sustainability attributes of no-till. Glob Chang Biol 26:3715–3725. https://doi.org/10.1111/gcb.15080

Cuypers A, Plusquin M, Remans T, et al (2010) Cadmium stress: An oxidative challenge. BioMetals 23:927–940. https://doi.org/10.1007/s10534-010-9329-x

Dejardin A, Rochat C, Wuilleme S, Boutin J-P (1997) Contribution of sucrose synthase, ADP-glucose pyrophosphorylase and starch synthase to starch synthesis in developing pea seeds. Plant, Cell Environ 20:1421–1430.

https://doi.org/10.1046/j.1365-3040.1997.d01-32.x

Desa (2015) World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241.

Embrapa (2020) Tecnologias de produção de soja, Sistemas de Produção 17. Londrina Embrapa Soja

Fageria NK, Stone LF (2008) Micronutrient deficiency problems in South America. In: Micronutrient deficiencies in global crop production. Springer, pp 245–266

Farias JRB, Nepomuceno AL, Neumaier N (2007) Ecofisiologia da soja. Embrapa Soja-Circular Técnica (INFOTECA-E)

Farmer EE, Mueller MJ (2013) ROS-Mediated Lipid Peroxidation and RES-Activated Signaling. Annu Rev Plant Biol 64:429–450. https://doi.org/10.1146/annurev-arplant-050312-120132

Farooq MA, Niazi AK, Akhtar J, et al (2019) Acquiring control: The evolution of ROS-Induced oxidative stress and redox signaling pathways in plant stress responses. Plant Physiol Biochem 141:353–369. https://doi.org/10.1016/j.plaphy.2019.04.039

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1977) Stage of soybean development. Spec Rep 80:929–931

Ferrari Neto J, Franzluebbers AJ, Crusciol CAC, et al (2021) Soil carbon and nitrogen fractions and physical attributes affected by soil acidity amendments under no-till on Oxisol in Brazil. Geoderma Reg 24:. https://doi.org/10.1016/j.geodrs.2020.e00347

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol 59:309–314. https://doi.org/https://doi.org/10.1104/pp.59.2.309

Gomes-Junior RA, Moldes CA, Delite FS, et al (2006) Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. Chemosphere 65:1330–1337. https://doi.org/https://doi.org/ 10.1016/j.chemosphere.2006.04.056.

Gómez R, Vicino P, Carrillo N, Lodeyro AF (2019) Manipulation of oxidative stress responses as a strategy to generate stress-tolerant crops. From damage to signaling to tolerance. Crit Rev Biotechnol 39:693–708. https://doi.org/10.1080/07388551.2019.1597829

Gratão PL, Monteiro CC, Antunes AM, et al (2008) Acquired tolerance of tomato (Lycopersicon esculentum cv. Micro-Tom) plants to cadmium-induced stress. Ann Appl Biol 153:321–333. https://doi.org/https://doi.org/ 10.1016/j.chemosphere.2006.04.056.

Grover SP, Butterly CR, Wang X, Tang C (2017) The short-term effects of liming on

organic carbon mineralisation in two acidic soils as affected by different rates and application depths of lime. Biol Fertil Soils 53:431–443. https://doi.org/10.1007/s00374-017-1196-y

Gupta A, Rico-Medina A, Caño-Delgado AI (2020) The physiology of plant responses to drought. Science (80-) 368:266–269. https://doi.org/10.1126/science.aaz7614

Hartman GL, West ED, Herman TK (2011) Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. Food Secur 3:5–17. https://doi.org/10.1007/s12571-010-0108-x

Heanes, D. L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Commun Soil Sci Plant Anal, 10, 191-1213.

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/https://doi.org/10.1016/0003-9861(68)90654-1.

Jahn R, Blume HP, Asio VB, et al (2006) Guidelines for soil description. FAO

Karr-Lilienthal LK, Grieshop CM, Merchen NR, et al (2004) Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. J Agric Food Chem 52:6193–6199. https://doi.org/10.1021/jf049795+

Kiehl EJ (1979) Edaphology manual: soil-plant relationship. Agronômica Ceres São Paulo, Brazil Piracicaba

Kleiner K, Abrams M, Schultz J (1992) The Impact of Water & Nutrient Deficiencies on the Growth. Tree Physiol. 11:271–287

Kopittke PM, Dalal RC, Menzies NW (2016) Sulfur dynamics in sub-tropical soils of Australia as influenced by long-term cultivation. Plant Soil 402:211–219

Krul ES (2019) Calculation of Nitrogen-to-Protein Conversion Factors : A Review with a Focus on Soy Protein. 339–364. https://doi.org/10.1002/aocs.12196

Lal R (2009) Soil degradation as a reason for inadequate human nutrition. Food Secur 1:45–57. https://doi.org/10.1007/s12571-009-0009-z

Li X, Lawas LMF, Malo R, et al (2015) Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress. Plant, Cell Environ 38:2171–2192. https://doi.org/10.1111/pce.12545

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Methods in enzymology. Elsevier, pp 350–382

Loix C, Huybrechts M, Vangronsveld J, et al (2018) Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. Front Plant

Sci 9:1–2. https://doi.org/10.3389/fpls.2018.00391

Moretti LG, Crusciol CAC, Bossolani JW, et al (2020) Bacterial Consortium and Microbial Metabolites Increase Grain Quality and Soybean Yield. J Soil Sci Plant Nutr 20:1935–1936. https://doi.org/10.1007/s42729-020-00263-5

Nora DD, Amado TJC (2013) Improvement in chemical attributes of Oxisol subsoil and crop yields under no-till. Agron J 105:1393–1403. https://doi.org/10.2134/agronj2013.0031

Nora DD, Amado TJC, Nicoloso R da S, et al (2017) Mitigation of the gradient of chemical properties in the rooting zone of dystrophic oxisols by gypsum and lime inputs under a no-till system. Rev Bras Cienc do Solo 41:1–22. https://doi.org/10.1590/18069657rbcs20150541

Paustian K, Babcock BA, Hatfield J, et al (2004) Climate change and greenhouse gas mitigation: challenges and opportunities for agriculture. CAST Task Force Rep 141:

Reid CD, Tissue DT, Fiscus EL, Strain BR (1997) Comparison of spectrophotometric and radioisotopic methods for the assay of Rubisco in ozone-treated plants. Physiol Plant 101:398–404. https://doi.org/https://doi.org/10.1111/j.1399-3054.1997.tb01014.x

Reis AR dos, Lisboa LAM, Reis HPG, et al (2018) Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. Plant Physiol Biochem 130:377–390. https://doi.org/10.1016/j.plaphy.2018.07.028

Rellán-Álvarez R, Lobet G, Dinneny JR (2016) Environmental Control of Root System Biology. Annu Rev Plant Biol 67:619–642. https://doi.org/10.1146/annurev-arplant-043015-111848

Ritchey KD, Feldhake CM, Clark RB, Sousa DMG (1995) Improved water and nutrient uptake from subsurface layers of gypsum-amended soils. In: Karlen DL et al. (ed) Agricultural Utilization of Urban and Industrial By-Products, (Ed.). ASA, CSSA, and SSSA, Madison, pp 157–181

Ritchey KD, Silva JE, Costa UF (1982) Calcium deficiency in clayey B horizons of savanna oxisols. Soil Sci 133:378–382

Rolim G de S, SENTELHAS PC, BARBIERI V (1998) Planilhas no ambiente EXCEL TM para os cálculos de balanços hídricos: normal, sequencial, de cultura e de produtividade real e potencial. Rev Bras Agrometeorol 6:133–137

Santos EF, Kondo Santini JM, Paixão AP, et al (2017) Physiological highlights of manganese toxicity symptoms in soybean plants: Mn toxicity responses. Plant Physiol Biochem 113:6–19. https://doi.org/10.1016/j.plaphy.2017.01.022

Shoemaker HE, McLean EO, Pratt PF (1961) Buffer Methods for Determining Lime Requirement of Soils With Appreciable Amounts of Extractable Aluminum. Soil Sci

Soc Am J 25:274–277. https://doi.org/10.2136/sssaj1961.03615995002500040014x

Silva FAM, Naudin K, Corbeels M, et al (2019) Impact of conservation agriculture on the agronomic and environmental performances of maize cropping under contrasting climatic conditions of the Brazilian Cerrado. F Crop Res 230:72–83. https://doi.org/10.1016/j.fcr.2018.10.009

Stein O, Granot D (2019) An overview of sucrose synthases in plants. Front Plant Sci 10:1–14. https://doi.org/10.3389/fpls.2019.00095

Sugiyama A, Ueda Y, Zushi T, et al (2014) Changes in the bacterial community of soybean rhizospheres during growth in the field. PLoS One 9:1–9. https://doi.org/10.1371/journal.pone.0100709

Tebebu TY, Bayabil HK, Steenhuis TS (2020) Can degraded soils be improved by ripping through the hardpan and liming? A field experiment in the humid Ethiopian Highlands. L Degrad Dev 31:2047–2059. https://doi.org/10.1002/ldr.3588

Thornthwaite CW, Mather JR (1955) The Water Balance Climatology. Laboratory of climatology, Philadelphia, PA: Drexel Institute of Technology

Unicamp (2020) Center of Meteorological and Climatic Research Applied to Agriculture. Botucatu: Municipalities climate of São Paulo State. www.cpa.unicamp.br/outras- informacoes/clima_muni_086.html

USDA (2014) Keys to soil taxonomy. US Gov. Print. Office, Washington, DC, pp 327–328

van Raij B, Cantarella H, Quaggio JA, Furlani AMC (1997) Recomendações de adubação e calagem para o Estado de São Paulo. Instituto Agronômico/Fundação IAC Campinas

van Raij B, Quaggio JA, Cantarella H, Abreu CA (2001) Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. Análise química para avaliação da Fertil solos Trop 5–39

Wang Q, Yang S, Wan S, Li X (2019) The significance of calcium in photosynthesis. Int J Mol Sci 20:1–14. https://doi.org/10.3390/ijms20061353

Zoca SM, Penn C (2017) An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. Adv Agron 144:1–44. https://doi.org/10.1016/bs.agron.2017.03.001

SUPPLEMENTARY MATERIAL

Table S1. Crops and treatments application scheme during the experimental period (from 2002 to 2019).

Growing season	Seasons		Treatments[†]
	Summer crops	Autumn-Winter-Spring crops	
2002/2003	*Oriza sativa*	*Avena strigosa*	L: 2.7 Mg ha^{-1} (71% ECCE[‡]) PG: 2.1 Mg ha^{-1}
2003/2004	*Phaseolus vulgaris*	*Avena strigosa*	-
2004/2005	*Arachis hypogaea*	*Avena sativa*	L: 2.0 Mg ha^{-1} (71% ECCE) PG: 2.1 Mg ha^{-1}
2005/2006	*Arachis hypogaea*	*Avena sativa*	-
2006/2007	*Zea mays* intercropped with *Urochloa brizantha* (cv. Marandu)	*Urochloa brizantha* (cv. Marandu) (after *Zea mays* harvest)	-
2007/2008	*Zea mays* intercropped with *Urochloa brizantha* (cv. Marandu)	*Urochloa brizantha* (cv. Marandu) (after *Zea mays* harvest)	-
2008/2009	*Glycine max*	*Avena strigosa*	-
2009/2010	*Glycine max*	*Sorghum vulgare*	-
2010/2011	*Zea mays*	*Crambe abyssinica* / *Vigna unguiculata*	L: 2.0 Mg ha^{-1} (88% ECCE) PG: 2.1 Mg ha^{-1}
2011/2012	*Zea mays*	*Crambe abyssinica* / *Vigna unguiculata*	-
2012/2013	*Pennisetum glaucum*	*Triticum aestivum*	-
2013/2014	*Phaseolus vulgaris*	*Triticum aestivum*	-
2014/2015	*Phaseolus vulgaris*	*Urochloa brizantha* (cv. Marandu) (1st growing season)	-
2015/2016	*Urochloa brizantha* (cv. Marandu) (2nd growing season)	*Urochloa brizantha* (cv. Marandu) (3rd growing season)	-
2016/2017[§]	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	L: 13.04 Mg ha^{-1} (69% ECCE) PG: 10 Mg ha^{-1}
2017/2018	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	-
2018/2019	*Glycine max*	-	-

[†]The reapplications in October 2004, 2010, and 2016 were performed when base saturation reached ≤ 50%; L = lime and PG = phosphogypsum.
[‡]ECCE: effective calcium carbonate equivalent
[§]Micronutrients applied in total area.

Table S2. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG)] for soil chemical properties in stratified layers (0.0–1.0 m depth).

Soil properties	Soil depth (m)					
	0.0–0.1	0.1–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
pH	<0.001	<0.001	0.0271	<0.001	0.0016	0.0048
CV† (%)	3.32	4.51	6.98	2.10	2.76	2.59
Ca^{2+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	4.20	5.22	5.87	5.01	9.12	5.80
Mg^{2+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	6.59	7.64	14.4	15.3	16.5	15.4
BS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	4.32	7.71	9.98	6.20	6.94	4.16
Al^{3+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	15.3	29.4	11.7	16.2	16.0	16.2
SO_4^{2-}-S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	10.6	12.0	12.5	11.0	9.87	13.7

Exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminum (Al^{3+}), base saturation (BS) and sulfate (SO_4^{2-}-S). †coefficient of variation (CV)

Table S3. Average and statistical parameters for soil organic matter (SOM), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) at 0.0–0.2 m depth affected by different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)].

Soil amendments (SA)	SOM	P	Fe	Mn	Cu	Zn
	g kg^{-1}	---------- mg kg^{-1} ----------				
Control	25.1 b	29.3 b	37.1 a	45.9 a	2.96 a	3.93 a
PG	25.0 b	29.2 b	34.4 b	35.8 b	2.72b	3.63 b
L	29.8 a	39.4 a	18.4 c	26.2 c	1.78 c	2.76 c
LPG	30.3 a	41.2 a	16.9 d	24.8 d	1.50 d	2.32 d
LSD†	1.59	4.14	1.47	2.54	0.07	0.28
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV‡ (%)	3.61	7.44	3.44	4.82	1.95	5.40

Different lower-case letters on the columns indicate significant differences between treatments by Student's t-test at $p \leq 0.05$. †Low significant difference (LSD), ‡coefficient of variation (CV). Soil organic matter (SOM), phosphorus (P) and available iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn).

Table S4. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for soybean root dry matter and root dry matter distribution in stratified layers (0.0–1.0 m depth).

Root parameters	Soil depth (m)					
	0.0–0.1	0.1–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
	Root dry matter					
Soil amendments (SA)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD†	0.0559	0.0297	0.0201	0.0157	0.0168	0.0138
Growing seasons (GS)	0.5624	0.5165	0.9984	0.9932	0.8325	0.9321
LSD	0.0568	0.0299	0.0234	0.0189	0.0178	0.0155
SA × GS	0.9778	0.9142	0.5699	0.9456	0.5256	0.8965
CV ‡ (%)	14.01	12.73	14.65	17.49	14.11	18.21
	Root dry matter distribution					
SA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	2.49	1.22	0.79	0.84	1.01	0.92
GS	0.2634	0.2354	0.0987	0.1235	0.1144	0.5463
LSD	3.23	1.65	0.98	0.79	0.88	0.81
SA × GS	0.5455	0.2451	0.0987	0.0752	0.4575	0.0889
CV (%)	3.8	2.45	3.68	7.18	14.16	14.79

†Low significant difference (LSD), ‡coefficient of variation (CV)

Table S5. Influence of lime (L) phosphogypsum (PG), and lime + phosphogypsum (LPG) on nutrient concentration in the leaves of soybean cultivated in two growing seasons in a long-term no-till system.

Nutritional plant status	units	Control	PG	L	LPG
N	g kg^{-1}	49.7 b	49.6 b	57.1 a	57.6 a
P	g kg^{-1}	3.15 b	3.39 a	3.45 a	3.45 a
K	g kg^{-1}	20.3 c	20.7 bc	22.8 a	21.4 b
Ca	g kg^{-1}	5.72 c	8.36 a	6.93 b	8.02 a
Mg	g kg^{-1}	3.26 c	2.19 d	3.92 b	4.43 a
S	g kg^{-1}	1.99 d	3.29 a	2.47 c	2.99 b
Fe	mg kg^{-1}	131 a	115 a	107 a	121 a
Mn	mg kg^{-1}	176 a	215 a	64.1 b	62.2 b
Cu	mg kg^{-1}	7.17 a	6.37 a	6.24 a	7.28 a
Zn	mg kg^{-1}	57.3 b	72.0 a	33.9 c	36.4 c

Different lower-case letters on the lines indicate significant differences between treatments by Student's t-test at $p \leq 0.05$. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), cupper (Cu) and zinc (Zn).

Table S6. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for nutritional status of soybean.

Treatments	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn
Soil amendments (SA)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.2631	<0.001	0.2414	<0.001
LSD†	1.73	0.08	0.92	0.57	0.25	0.27	24.8	39.3	1.28	9.47
Growing seasons (GS)	<0.001	<0.001	<0.001	0.0057	0.0015	0.0203	0.4094	0.4339	0.4243	0.2487
LSD	1.22	0.06	0.65	0.40	0.18	0.19	17.5	27.8	0.91	6.70
SA × GS	0.9679	0.9719	0.6346	0.9644	0.7827	0.9794	0.9129	0.9807	0.9140	0.9620
CV ‡ (%)	3.10	2.33	4.16	7.56	6.95	9.49	20.1	29.3	18.2	18.3

Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). †Low significant difference (LSD), ‡coefficient of variation (CV).

Table S7. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for soybean physiological, biochemical and agronomic parameters.

Treatments	Chl *a*	Chl *b*	Total chl	Carotenoids	*A*	*gs*	*E*	*ic*	WUE	Rubisco
Soil amendments (SA)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Growing seasons (GS)	<0.001	0.0256	<0.001	0.4173	<0.001	<0.001	<0.001	<0.001	<0.001	-
SA × GS	0.0467	0.0385	0.0464	0.0456	0.0452	0.0113	0.0493	0.0030	0.0042	-
LSD† (SA × GS)	1.81	0.84	2.65	0.49	1.20	0.04	0.89	4.78	0.90	0.13
CV‡ (%)	10.7	18.4	11.55	10.1	5.26	14.9	7.9	6.16	10.8	4.71
	Sucrose	Susy	H_2O_2	MDA	SOD	CAT	APX	GR	SDM	GY
SA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GS	<0.001	-	<0.001	0.0198	<0.001	0.0167	0.5930	<0.001	<0.001	<0.001
SA × GS	<0.001	-	0.0324	0.0366	0.0487	0.0376	<0.001	0.0347	<0.001	0.0379
LSD (SA × GS)	1.75	2.21	0.65	1.05	1.10	0.55	0.63	0.07	0.34	0.15
CV (%)	3.46	9.76	6.41	7.34	5.71	9.83	12.0	26.8	6.87	6.40

Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), total chlorophyll (Total chl), carotenoids (Carot), Net photosynthesis rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*ic*), water use efficiency (WUE), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), shoot dry matter (SDM) and grain yield (GY). †Low significant difference (LSD), ‡coefficient of variation (CV).

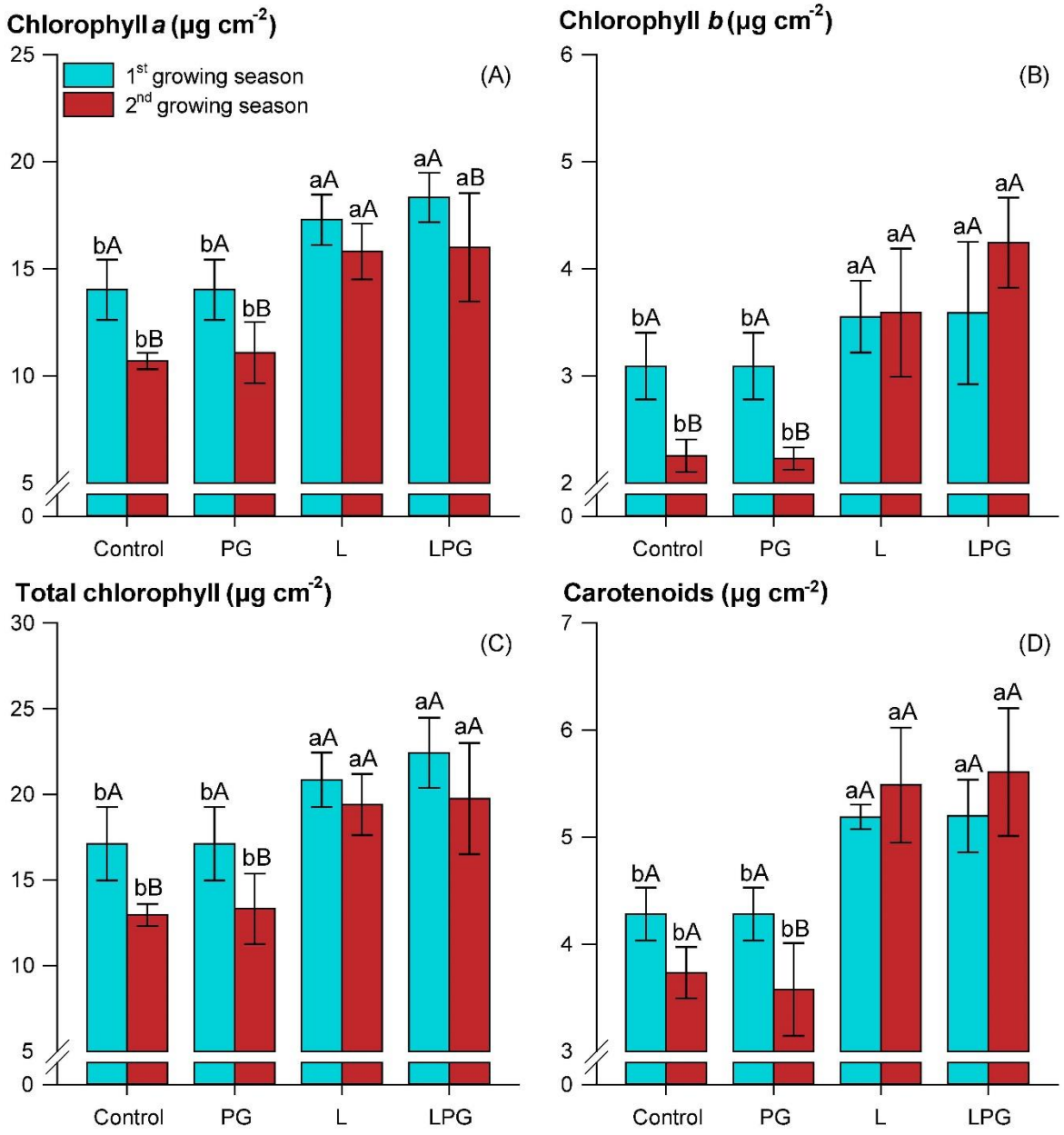


Figure S1. Effects of the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] on (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) total chlorophyll, and (D) carotenoids concentrations in soybean leaves. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$)

CHAPTER 2

LONG-TERM LIME AND PHOSPHOGYPSUM AMENDED-SOILS ALLEVIATES THE FIELD DROUGHT EFFECTS ON CARBON AND ANTIOXIDATIVE METABOLISM OF MAIZE BY IMPROVING SOIL FERTILITY AND ROOT GROWTH[2]

Published in: Bossolani, J.W., Crusciol, C.A.C., Garcia, A., Moretti, L.G., Portugal, J.R., Rodrigues, V.A., Fonseca, M. de C. da, Calonego, J.C., Caires, E.F., Amado, T.J.C., Reis, A.R. dos, 2021a. Long-term lime and phosphogypsum amended-soils alleviates the field drought effects on carbon and antioxidative metabolism of maize by improving soil fertility and root growth. Front. Plant Sci. 12, 1437. https://doi.org/10.3389/fpls.2021.650296

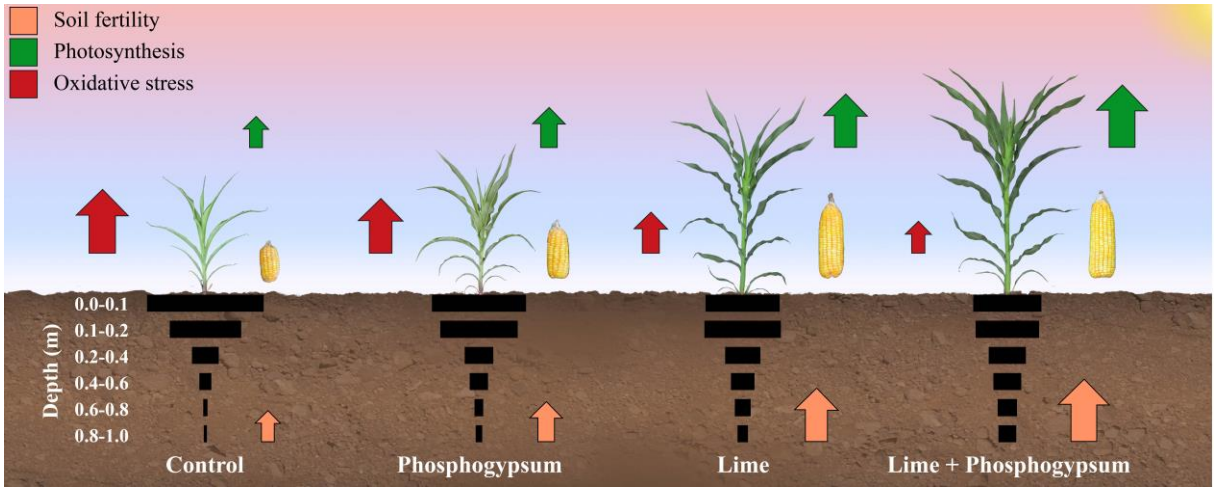
Abstract

Long-term surface application of lime (L) and/or phosphogypsum (PG) in no-till (NT) systems can improve plant growth and physiological and biochemical processes. Although numerous studies have examined the effects of L on biomass and plant growth, comprehensive evaluations of the effects of this practice on net CO_2 assimilation, antioxidant enzyme activities and sucrose synthesis are lacking. Accordingly, this study examined the effects of long-term surface applications of L and PG on soil fertility and the resulting impacts on root growth, plant nutrition, photosynthesis, carbon and antioxidant metabolism, and grain yield of maize established in a dry winter region. At the study site, the last soil amendment occurred in 2016, with the following four treatments: control (no soil amendments), L (13 Mg ha^{-1}), PG (10 Mg ha^{-1}), and L and PG combined (LPG). The long-term effects of surface liming included reduced soil acidity and increased the availability of P, Ca^{2+} and Mg^{2+} throughout the soil profile. Combining L with PG strengthened these effects and also increased SO_4^{2-}-S. Amendment with LPG increased root development at greater depths and improved maize plant nutrition. These combined effects increased the concentrations of photosynthetic pigments and gas exchange even under low water availability. Furthermore, the activities of Rubisco, sucrose synthase and antioxidative enzymes were improved, thereby reducing oxidative stress. These improvements in the physiological performance of maize plants led to higher grain yield. Overall, the findings support combining soil amendments as an important strategy to increase soil fertility and ensure crop yield in regions where periods of drought occur during the cultivation cycle.

Keywords: Soil fertility, Soil amendments, Root distribution, Rubisco, Sucrose synthase, Oxidative stress

[2] Chapter written in accordance with the rules of Frontiers in Plant Science.

Graphical abstract



2.1 INTRODUCTION

Soil degradation is a serious threat to food security worldwide (Bindraban et al., 2012; FAO, 2015; Gibbs and Salmon, 2015). Global drivers of soil degradation include erosion processes, contamination by heavy metals and other toxic substances, and soil acidification (FAO, 2015). Globally, ~2.0 billion hectares (ha) of arable soil in the tropics is affected by high acidity (Bian et al., 2013), which is the most important factor limiting crop development and food production capacity in tropical crop systems (Costa and Crusciol, 2016). Acidic and weathered soils are naturally less fertile due to the limited availability of calcium (Ca^{2+}), magnesium (Mg^{2+}) and phosphorus (P) and high aluminum (Al^{3+}) availability, especially in the deepest soil layers (Nora et al., 2017; Costa et al., 2018). Low nutrient availability coupled with high Al^{3+} levels leads to root growth inhibition and, consequently, decreased water and nutrient uptake, resulting in physiological dysfunction and lower yields (Eekhout et al., 2017; Reis et al., 2018). In addition, tropical regions are subject to periods of drought stress, mainly during the autumn/winter seasons (mid-March to September). The combination of low soil fertility and limited root development with prolonged periods of water limitation contributes strongly to lower yields, especially since the vast majority of the cultivated area is in upland conditions (Carmeis-Filho et al., 2017). While advances in plant biotechnology have aided the development of plants that are more resistant to abiotic stresses (Gupta et al., 2020), soil management practices that enable crop development even under unfavorable conditions remain fundamental to food production (Gibbons et al., 2014;

Holland et al., 2018).

Several management measures have been developed to mitigate the hazardous effects of soil acidification and improve and sustain soil productivity. In particular, liming has become a widespread practice to raise fertility levels and restore soil quality (Holland et al., 2018; Bossolani et al., 2020a, 2020b, 2021a). Liming aims to increase soil pH, which in turn improves effective cation exchange capacity (ECEC) and base saturation (BS) and reduces Al^{3+} and manganese (Mn) concentrations (Crusciol et al., 2019; Bossolani et al., 2020a). However, due its low solubility, the effects of lime occur mostly in the top layers, mainly after surface applications under no-till (NT), and more slowly in deep (Caires et al., 2011). Amendment with phosphogypsum (PG) can also improve soil fertility but cannot correct soil acidity (Zoca and Penn, 2017). PG application increases Ca^{2+} and sulfate (SO_4^{2-}) levels throughout the soil profile, which reduces Al^{3+} toxicity due to $AlSO_4^{+}$ ion pair formation and decreased Al^{3+} saturation (Caires et al., 2011; Zoca and Penn, 2017). These changes enhance root development in deeper soil layers (Costa et al., 2018), increasing crop tolerance to water limitation (Costa and Crusciol, 2016; Nora et al., 2017). Thus, the surface application of PG is an important complementary strategy to overcome the surface liming limitations (Bossolani et al., 2018, 2020b; Crusciol et al., 2019).

Several types of abiotic stress can negatively affect the photosynthetic processes of plants and generate oxidative stress, with deleterious effects on key cellular components and functions (Gómez et al., 2019). Crops established in fertile soils tend to have a greater ability to maintain relatively high levels of growth, stomatal conductance, photosynthesis, and antioxidant metabolism under environmental stresses (e.g., drought) (Kleiner et al., 1992). In addition, less stressed plants exhibit delayed senescence, thereby further increasing photosynthetic capacity and enabling high yields (Gómez et al., 2019). In the present study, a detailed investigation was performed to determine (1) how amending soil with lime and PG under NT influences soil chemical properties and induces improvements in maize root development, nutrient uptake, physiology and yield and (2) the main changes in the soil and crop nutrition that alter carbon and antioxidant metabolism in maize plants under field conditions.

2.2 MATERIAL AND METHODS

2.2.1 Site description, experimental design and treatments

The study site was a long-term field experiment (registered on the GLTEN Metadata Portal; https://www.glten.org/experiments/62) with lime and PG application that has been carried out in Botucatu, southeastern São Paulo State, Brazil (22° 83' 3" S, 48° 42' 64" W, 765 m above sea level), under NT since 2002. The soil was classified as a sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2014). Prior to the beginning of the experiment in 2002, soil granulometric and chemical properties (0.0–0.2 m depth) were determined according to the methods of Kiehl (1979) and van Raij et al. (2001), respectively, as follows: soil pH (0.01 M $CaCl_2$ suspension): 4.2; soil organic carbon (SOC): 12.2 g kg^{-1}; P (resin): 9.2 mg kg^{-1}; exchangeable K^+: 1.2 $mmol_c$ kg^{-1}; exchangeable Ca^{2+}: 14 $mmol_c$ kg^{-1}; exchangeable Mg^{2+}: 5 $mmol_c$ kg^{-1}; total acidity at pH 7 (H+Al): 37 $mmol_c$ kg^{-1}, cation exchange capacity (CEC): 57.2 $mmol_c$ kg^{-1}; BS: 35%; aluminum saturation (AS): 65%; sand: 540 g kg^{-1}; silt: 110 g kg^{-1}; and clay: 350 g kg^{-1}. In addition, the clay content at 0.2–0.4 m depth was 360 g kg^{-1}. According to Köppen-Geiger's climate classification (Alvares et al., 2013), the region is a mesothermic type (Cwa) with a humid subtropical climate, dry winters and hot summers. For the period 1956-2019, the average annual maximum and minimum temperatures were 26.1°C and 15.3°C, respectively, and the average annual pluvial precipitation was 1,360 mm (Unicamp, 2020).

The experimental design was a randomized complete block with four treatments and four replicates. The treatments were composed by: (*i*) control (16 years without soil amendments with intensive crop cultivation and fertilizer inputs); (*ii*) exclusive application of PG; (*iii*) exclusive application of lime (L); and (*iv*) combined application of L and PG (LPG). Liming occurred by application of sedimentary dolomitic lime [$CaMg(CO_3)_2$] (www.calcarioguapirama.com.br), with 233 g kg^{-1} CaO and 175 g kg MgO. The PG contained 280 g kg^{-1} CaO, 150 g kg^{-1} S, <1 g kg^{-1} P, and <1 g kg^{-1} fluorine (F). The soil amendments were applied in 2002, 2004, 2010, and 2016 based on the results of annual sampling. The criterion for reapplying the treatments was BS ≤ 50% in the L treatment. The lime rate was calculated to increase the BS in the topsoil (0.0–0.2 m) to 70% according to the criterion proposed by van Raij et al. (1997) and was 2.7 Mg ha^{-1} in 2002 and 2.0 Mg ha^{-1} in 2004 and 2010. In October 2016, a new

application was necessary, however the lime rate calculation method used was revised considering the layer of 0.0–0.4 m depth, and then, multiplying by a factor 2 (double of the recommended dose), resulting in 13 Mg ha^{-1} of L. When lime reapplication was required, PG was also reapplied. In 2002, 2004 and 2010, the rates of PG application were determined according to van Raij et al. (1997) by multiplying the clay content in the 0.2–0.4 m layer by a factor of 6, resulting in a rate of 2.1 Mg ha^{-1}. In 2016, the most recent methodology for PG application in tropical soils proposed by Caires and Guimarães (2018), which is intended to increase Ca^{2+} saturation in the ECEC to 60% in the 0.2–0.4 m soil layer, was used, resulting in a higher PG rate of 10 Mg ha^{-1}. After the last soil amendments reapplication, a micronutrient based fertilizer was applied over a total area at rates of 3 kg ha^{-1} B + 1 kg ha^{-1} Cu + 1 kg ha^{-1} Mn + 10 kg ha^{-1} Zn + 0.2 kg ha^{-1} Mo, in order to avoid unavailability of micronutrients due to the increase in pH by liming. This study characterizes the long-term residual effects of these treatments in the first and second years after the last soil amendment reapplications in 2016. Several crops were grown during the agricultural years from 2002 to 2018. Previous crops grown and details of the L and PG applications are shown in Table S1. Except for the first applications of lime and PG in 2002, when the experiment under NT began, all applications were applied to the soil surface.

2.2.2 Crop management

Maize (simple hybrid P3707VYH; 60,000 plants ha^{-1}; DuPont Pioneer®, Johnston, IA,USA) was sown in March 2017 and 2018. For both growing seasons, fertilization was performed at sowing with 28 kg ha^{-1} N, 98 kg ha^{-1} P_2O_5, and 56 kg ha^{-1} K_2O. In addition, a topdressing fertilization with 90 kg N ha^{-1} $(NH_4)_2SO_4$ (Cantarella et al., 1997) as ammonium sulfate was performed at the V_4 phenological stage (Ritchie et al., 1993). The field plots consisted of 14 rows with a length of 9 m spaced 0.45 m apart (56.7 m^2). The plots were spaced 8 m from each other to avoid cross-contamination from surface runoff containing fertilizers as a consequence of heavy rainfall or during treatment applications and sowing.

2.2.3 Meteorological data

Throughout the experimental period, meteorological data (rainfall, solar

radiation, wind speed, relative humidity and maximum and minimum temperatures) were obtained through automatic meteorology station installed close to the experimental area. The climatological water balance of the two growing seasons is shown in Fig. 1. The evapotranspiration reference (ET_0) was calculated using the Penman-Monteith method (Allen et al., 1998). The crop evapotranspiration (ETc) was calculated using the crop coefficient (Kc) for each stage of the crop's phenological stage (Allen et al., 1998). Using the rainfall data, the climatological water balance was monitored and calculated using electronic spreadsheets (Rolim et al., 1998), following the procedure of Thornthwaite and Mather (1955) to determine the real evapotranspiration (ETr).

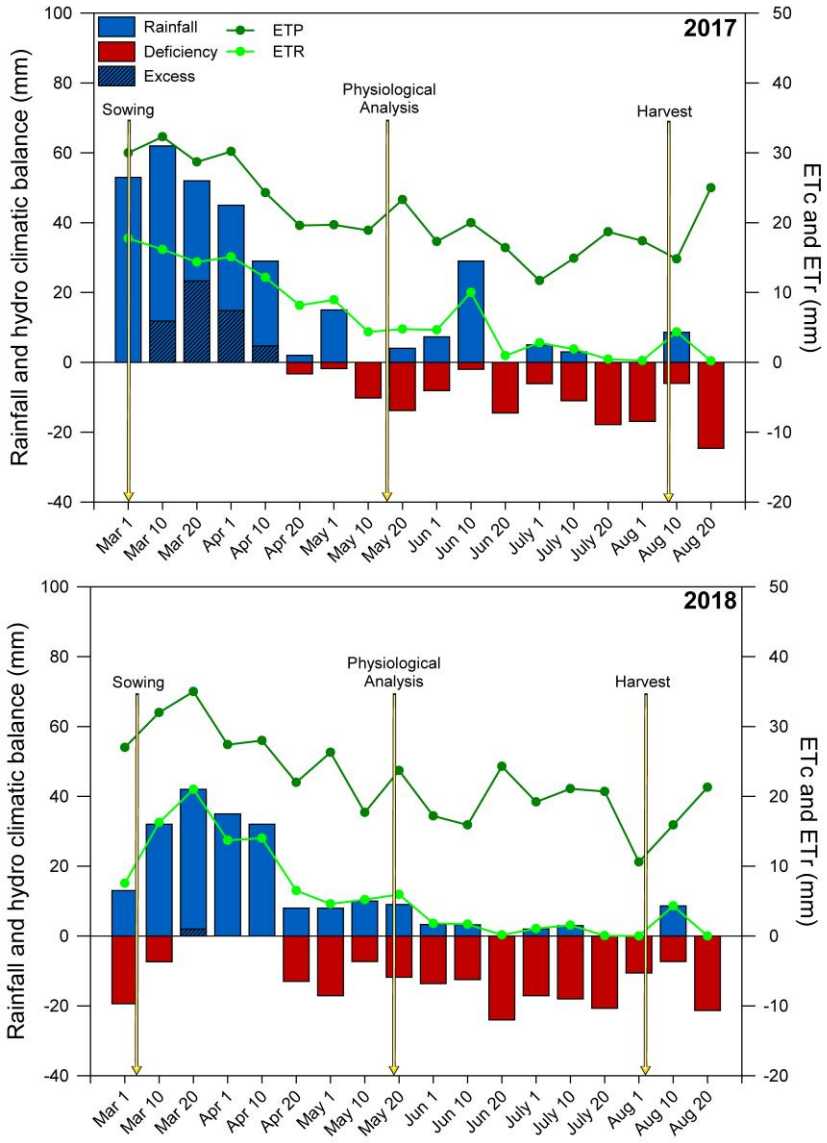


Figure 1. Climatological water balance at Botucatu-SP, Brazil, during the maize crop

cycle. ETc, crop evapotranspiration; ETr, real evapotranspiration. The arrows indicate the managing and sampling time.

2.2.4 Soil chemical properties analysis

In October 2018 (24 months after the last reapplication of soil amendments), eight individual soil subsamples were randomly taken at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m in each plot, except for SOC, P, Fe, Mn, Cu, and Zn, that were sampled at a depth of 0.0–0.2 m. The samples were dried, sieved (2 mm mesh) and analyzed according to van Raij et al. (2001). The soil chemical analysis included soil pH (0.01 M $CaCl_2$), soil organic carbon (SOC) (Heanes, 1984), exchangeable cations (K^+, Ca^{2+}, and Mg^{2+}) and P extracted by ion-exchange resin and determined by atomic absorption spectrophotometry (AAS) and colorimetric method (van Raij et al., 2001), respectively. Total acidity (H+Al) was estimated using Smith-McLean-Pratt (SMP) solution (Shoemaker et al., 1961), whereas exchangeable Al^{3+} was extracted using 1 M KCl and both determined by titration with 0.025 M NaOH solution. SO_4^{2-}-S content was extracted by 0.01 M calcium phosphate solution (Bardsley and Lancaster, 1960), and determined by a turbidimetric method. Cationic micronutrients (Fe, Mn, Cu and Zn) were extracted with a solution containing 0.005 M diethylenetriaminepentaacetic acid (DTPA) pH 7.3, 0.1 M triethanolamine (TEA) and 0.01 M $CaCl_2$ and determined by AAS (van Raij et al., 2001).

2.2.5 Root sampling and dry matter determination

In both growing seasons, at maize full flowering (Ritchie et al., 1993), eight root subsamples (four subsamples from the plant rows and four subsamples from the middle of the inter-rows) were collected randomly from each plot and combined. A galvanized steel probe with a 82-mm-diameter cutting tip was used at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m. Roots were carefully separated from soil and other residues by washing under a flow of swirling water over a 0.5-mm mesh sieve. The samples were dried in a forced-air oven at 60°C for 72 h to measure root dry matter, expressed in g m^{-3} and subsequently estimated to Mg ha^{-1} in the 0.0–1.0 m layer. The root dry matter distribution was calculated from the ratio of root dry matter in each layer to total root dry matter.

2.2.6 Leaves sampling for crop nutrition and physiologic analysis

Nutritional, physiological and biochemical analyses occurred on the same diagnostic leaves, when maize plants were at the full flowering (R_2 phenological stage) (Ritchie et al., 1993). The diagnostic leaves selected were the fully expanded leaves in the top third of the maize canopy. Leaves sampling occurred immediately after the gas exchange analyses. Nutritional analysis occurred in dried and milled plant material, whereas the leaves for physiological analysis were placed in liquid nitrogen and stored at -80 °C until further analysis.

Nutritional determination

Leaf N was extracted by sulfuric digestion and determined by Kjeldahl method. Phosphorus, K, Ca, Mg, sulfur (S), Fe, Mn, Cu, and Zn extraction occurred by nitroperchloric digestion, and determined by AAS. Both methods are described by AOAC (2016).

Gas exchange parameters

Gas exchange assessments consisted of non-destructive analyses of fifteen diagnostic maize leaves using a using a Portable Infrared Gas Analyzer CIRAS-3 Portable Photosynthesis System (PP Systems Inc., Amesbury, MA, USA). The following parameters were determined on the diagnostic leaves of maize: net photosynthetic rate expressed as area (*A*; μmol CO_2 m^{-2} s^{-1}); stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}); internal CO_2 concentration in the substomatal chamber (*ic*; mmol CO_2 mol^{-1} air); leaf transpiration (*E*; mmol H_2O m^{-2} s^{-1}); and water use efficiency (WUE; μmol CO_2 (mmol H_2O)$^{-1}$), calculated by the *A*/*E* ratio. Readings began after the air temperature in the chamber was adjusted to 28°C, with 380 ppm CO_2 and 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) supplied by LED lamps. The measurements were performed between 8:00 and 10:00 a.m. The minimum equilibration time before performing the reading was 3 min.

Photosynthetic pigments

To determine photosynthetic pigments (chlorophyll *a*, *b*, total chlorophyll and total carotenoids), five discs were cut between the edge and central rib of maize leaves using a paper punch (0.5 cm in diameter). The discs were stored for 24 h in capped glass vials wrapped in aluminum foil and containing 2 mL of N, N-dimethylformamide

(DMF) according to the methodology proposed by Lichtenthaler (1987). The pigment concentrations were quantified in a spectrophotometer at wavelengths of 664, 647, and 480 nm for chlorophyll *a*, *b* and carotenoids, respectively. The absorbance was read after mixing 1 mL of the extract with 1 mL of distilled water. The calculations for the pigment concentrations were in accordance with the methods proposed by Wellburn (1994).

Ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Rubisco, EC 4.1.1.39)

Total Rubisco activity (determined only in the second growing season) was measured according to the method described by Reid et al. (1997). Frozen plant material (0.3 g) was ground with a mortar and pestle under liquid nitrogen and suspended in extraction buffer containing 1.5 mL of 58 mM potassium phosphate and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenized material was centrifuged at 14,000 rpm for 25 min at 4 °C, and the supernatant was stored at 4°C (adapted from Sage, Sharkey and Seemann 1988; Reid et al. 1997). The Rubisco incubation buffer contained 100 mM bicine-NaOH pH 8.0, 25 mM potassium bicarbonate ($KHCO_3$), 20 mM magnesium chloride ($MgCl_2$), 3.5 mM ATP, 5 mM phosphocreatine, 0.25 mM NADH, 80 nkat glyceraldehyde-3-phosphate dehydrogenase, 80 nkat 3-phosphoglyceric phosphokinase and 80 nkat creatine phosphokinase. A 70-µL aliquot of the supernatant was incubated with 900 µl of the incubation buffer at 30°C for 5 min in the absence of ribulose-1,5-bisphosphate (RuBP) to enable carbamylation of Rubisco. NADP oxidation was initiated by adding 30 µL of 16.66 mM RuBP directly into the cuvette. Readings were obtained in a spectrophotometer at a wavelength of 340 nm. Rubisco activity was calculated from the difference in the absorbance readings at 0 and 1 min (without removing the cuvette from the spectrophotometer) and expressed in µmol min^{-1} mg $protein^{-1}$.

Sucrose synthase activity (Susy, EC 2.4.1.13)

To extract sucrose synthase (Susy) (determined only in the second growing season), 0.5 g of frozen plant material was ground with a mortar and pestle under liquid nitrogen and suspended in extraction buffer containing 50 mM HEPES buffer pH 7.0, 2 mM $MgCl_2$, 2 mM dithiothreitol (DTT) and 1 mM EDTA (Dejardin et al., 1997). The homogenized material was centrifuged at 14,000 rpm for 20 min at 4°C, and the supernatant was stored at 4°C. The incubation buffer consisted of 0.1 M MES buffer

pH 6.0, 5 mM $MgCl_2$, 0.3 M sucrose and 5 mM uridine 5'-trihydrogen diphosphate (UDP). A 0.5-mL aliquot of the supernatant was incubated with 3.5 mL of incubation buffer at 37°C for 30 min, and the reaction was terminated by adding 100 µL of potassium hydroxide (30% KOH; w/v) and heating at 100°C for 5 min. Readings were obtained in a spectrophotometer at a wavelength of 540 nm, and the results were expressed as µmol sucrose g^{-1} fresh weight (FW) h^{-1}.

Sucrose concentration

The sucrose concentration was determined from 1 g of frozen plant material extracted in 10 mL of MCW solution (60% methanol, 25% chloroform, and 15% water; (w/v)) after maceration by a mortar and pestle. The homogenized material was centrifuged at 8,000 rpm for 10 min at 4°C. An aliquot of 4 mL of the supernatant was removed and mixed with 1 mL of chloroform and 1.5 mL of distilled water in another tube. After separation of the phases, the sucrose content was determined in the aqueous phase as described by Bieleski and Turner (1966). In brief, a 50-µL aliquot of the aqueous phase was mixed with 500 µL of 30% KOH (w/v) and 2 mL of H_2SO_4. After homogenization by a vortex mixer, the tube was heated at 100°C for 10 min. After cooling, readings were obtained in a spectrophotometer at a wavelength of 490 nm. The sucrose concentration was determined by reference to a standard sucrose curve and expressed as mg g^{-1} FW.

Hydrogen peroxide and lipid peroxidation

The hydrogen peroxide (H_2O_2) concentration was determined according to the methodology proposed by Alexieva et al. (2001). Frozen plant material (0.4 g) was ground with a mortar and pestle under liquid nitrogen, and 4 mL of 0.1% trichloroacetic acid (TCA) was added as extraction buffer. The homogenized material was centrifuged at 12,000 rpm for 15 min at 4°C. An aliquot of 200 µL of supernatant was mixed with 200 µL of 100 mM potassium phosphate buffer (pH 7.5) and 800 µL of 1 M potassium iodide and incubated at 1°C for 1 h. After warming to room temperature, readings were obtained in a spectrophotometer at a wavelength of 390 nm. The leaf concentration of H_2O_2 was determined by reference to a standard curve and expressed as µmol g^{-1} FW. Lipid peroxidation, or malondialdehyde (MDA), was measured according to the methodology proposed by Heath and Packer (1968). The extraction was carried out using 0.4 g of frozen plant material ground with a mortar and pestle under liquid

nitrogen and suspended in 4 mL of 0.1% (w/v) TCA + 20 % (w/v) polyvinylpolypyrrolidone (PVPP). The homogenized material was centrifuged at 10,000 rpm for 15 min at 4°C. A 250-µL aliquot of supernatant was mixed with 1 mL of 20% TCA + 0.5% thiobarbituric acid (w/v) solution to start the reaction. The reactions were heated at 95°C for 30 min and then placed on ice for 10 min. The reactions were centrifuged for 10 min at 10,000 rpm, and readings of the supernatant were obtained in a spectrophotometer at wavelengths of 535 and 600 nm. The results were expressed as nmol MDA g^{-1} FW.

Soluble protein

Proteins were extracted from 1.5 g of frozen plant material ground with a mortar and pestle under liquid nitrogen and suspended in 20% PVPP and extraction buffer containing 100 mM potassium phosphate pH 7.5, 1 mM EDTA, and 1 mM DDT. The homogenized material was centrifuged at 10,000 rpm for 25 min at 4°C, and the supernatant was stored in Eppendorf tubes in a freezer at -80ºC. The soluble protein concentration was determined using BSA (bovine serum albumin) as a standard according to the method proposed by Bradford (1976). Aliquots of 100 µL of the protein extract were mixed with 5 mL of Bradford reagent and analyzed in a spectrophotometer at a wavelength of 595 nm. The results and protein extract were used to determinate the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR).

Superoxide dismutase (SOD, EC:1.15.1.1)

SOD activity was determined according to Giannopolitis and Ries (1977). The reaction was conducted in a reaction chamber under illumination with a 15-W fluorescent lightbulb at 25ºC after adding 2 mL of 50 mM potassium phosphate buffer pH 7.8, 250 µL of 13 mM methionine, 200 µL of 75 mM nitroblue tetrazolium (NBT), 200 µL of 0.1 mM EDTA and 250 µL of 2 µM riboflavin to 50 µL of protein extract. The tubes were vortexed and placed inside the chamber (total absence of ambient light) for 15 min top permit the formation of the blue formazan compound by photoreaction of NBT. A control was prepared for each sample using the same conditions, except that the tubes were covered with aluminum foil to prevent light exposure. After 15 min, the reactions were vortexed, and readings were obtained in a spectrophotometer at a wavelength of 560 nm. The results were expressed as U (unit) SOD mg^{-1} protein.

Catalase (CAT, 1.11.1.6)

Catalase activity was determined by monitoring the degradation of H_2O_2 according to the methodology proposed by Azevedo et al. (1998). A 25-µL aliquot of protein extract was added to a mixture containing 1 mL of 100 mM potassium phosphate buffer pH 7.5 and 2 µL of 30% H_2O_2 (v/v) in a test tube and mixed quickly by vortexing. Enzyme activity was determined by the decomposition of H_2O_2 during a 2-min interval as measured using a spectrophotometer at a wavelength of 240 nm. The results were expressed as µmoL min^{-1} mg^{-1} protein.

Ascorbate peroxidase (APX, EC:1.11.1.11)

Ascorbate peroxidase activity was determined according to Gratão et al. (2008). A 100-µL aliquot of protein extract was added to 600 µL of 80 mM potassium phosphate buffer pH 7.0, 100 µL of 5 mM ascorbate and 100 µL of 1 mM EDTA and homogenized by vortexing. The mixture was incubated for 5 min at 30°C in the dark. A 100-µL aliquot of 1 mM H_2O_2 was then added, and readings were obtained immediately in a spectrophotometer at a wavelength of 290 nm for 2 min. The results were expressed as µmol min^{-1} mg^{-1} protein.

Glutathione reductase (GR, EC 1.6.4.2)

Glutathione reductase activity was determined according to the methodology described by Gomes-Junior et al. (2006). One milliliter of 100 mM potassium phosphate buffer pH 7.5, 500 µL of 1 mM nitrobenzoic acid (DTNB), 100 µL of 1 mM oxidized glutathione (GSSG) and 100 µL of 0.1 mM NADPH were added to an Eppendorf tube and mixed thoroughly. A 50-µL aliquot of the supernatant was added and vortexed. The solution was then transferred to a cuvette, and the absorbance at 412 nm was immediately recorded for 2 min. The results were expressed as µmol min^{-1} mg^{-1} protein.

2.2.7 Shoot dry matter and grain yield of maize

Maize shoot dry matter and grain yield were evaluated at the harvest stage based on the relationship between the masses (shoot and grain) obtained in the interior (5.4 m^2) of each plot and their respective water contents (0 and 130 g kg^{-1} of water for

shoot dry matter and grain yield, respectively).

2.2.8 Statistical analysis

Means were subjected to tests of homoscedasticity followed by the Anderson-Darling test of normality (Nelson, 1998). To evaluate homogeneity, Levene's test in the Minitab statistical program was used. Subsequently, the means were subjected to analysis of individual variance (ANOVA) by the F test ($p \leq 0.05$) and, when significant, analyzed using the modified t-test (Fisher's protected least significant difference (LSD) at $p \leq 0.05$). Redundancy analysis (RDA) was performed to determine the correlations among soil fertility × crop nutrition (average of two growing seasons), soil fertility × crop physiology (average of two growing seasons), and crop nutrition × crop physiology. The Monte Carlo permutation test was applied with 999 random permutations to verify the significance of soil chemical properties, crop nutrition and for physiological responses. One-way PERMANOVA (Anderson, 2005) was used to group treatments by similarity. Heatmaps were constructed by calculating the Pearson's correlation coefficients ($p \leq 0.05$), and only significant correlations are shown.

2.3 RESULTS

2.3.1 Weather conditions

In the first and second growing seasons, maize received 315 and 210 mm of pluvial precipitation, respectively (Fig. 1). From early-March (maize sowing) until early July (maize physiological maturity) of each year, only small amounts of rain occurred, resulting in a negative hydric balance.

2.3.2 Soil fertility and root development

Twenty-four months after the last reapplication of soil amendments, significant variations ($p < 0.01$; Table S2) in soil chemical properties among the treatments were observed in all layers of the soil profile (Fig. 2). Surface amendment with L (regardless of PG addition) increased the soil pH in all soil layers (0.0–1.0 m); additionally, acidity neutralization was not improved by PG application compared with the control (Fig. 2A).

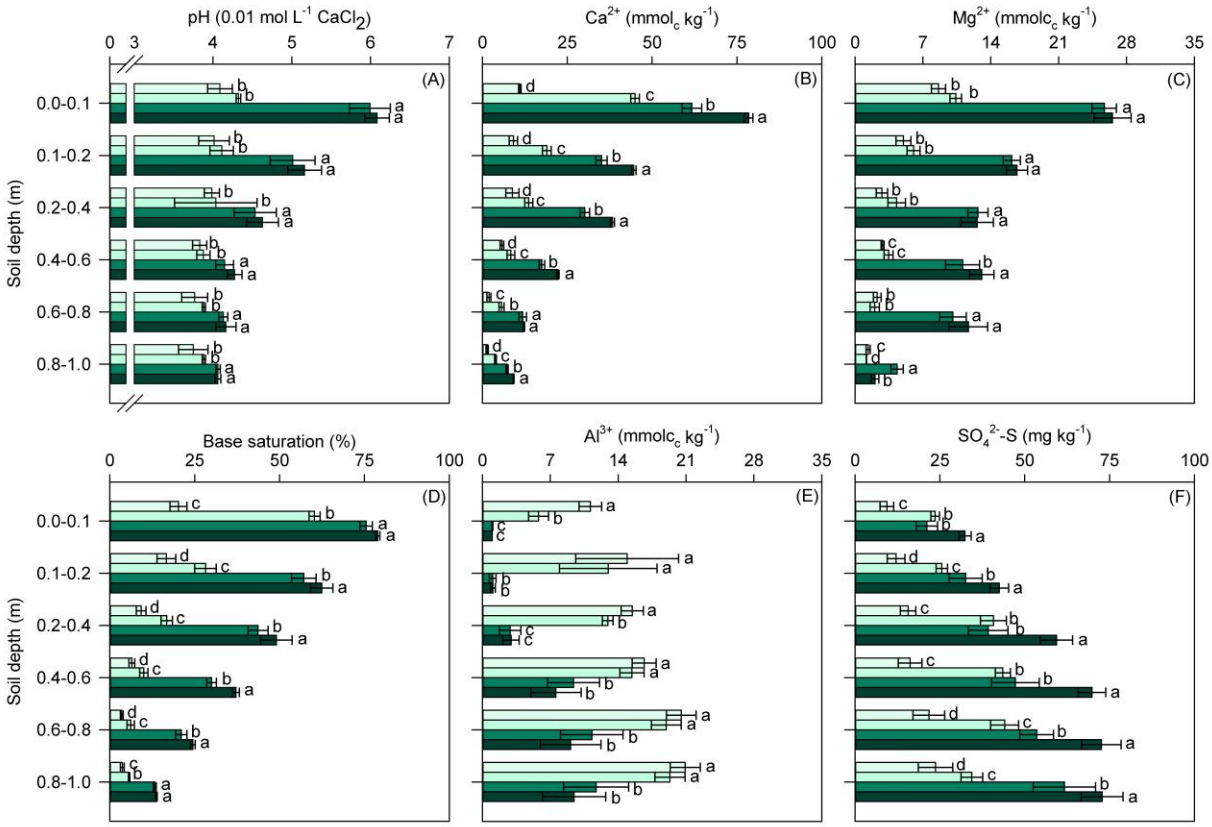


Figure 2. Changes in soil pH (A), exchangeable calcium (Ca^{2+}) (B) and magnesium (Mg^{2+}) (C), base saturation (BS) (D), exchangeable aluminum (Al^{3+}) (E) and sulfate (SO_4^{2-}-S) (F) in the soil profile as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters for each soil depth indicate significant differences between treatments by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

By contrast, significant increases in Ca^{2+} availability occurred in all soil layers when soil was amended with L and, in particular, LPG (Fig. 2B). PG increased the levels of Ca^{2+} in topsoil in relation to the control, resulting in high BS values (Fig. 2D), but BS remained lower in the PG treatment than in the L and LPG treatments. Mg^{2+} availability did not differ between L and LPG, but was higher in both of these treatments than in the control and PG treatments (Fig. 2C). BS was slightly improved by LPG compared with L alone, mainly in deeper soil layers (0.1 to 0.8 m) (Fig. 2D).

Aluminum availability was strongly reduced by the application of L and LPG, mainly in the uppermost layers (0.0–0.4 m depth); in soil depths below 0.4 m, there was no difference in aluminum availability between L and LPG (Fig. 2E). In addition, the application of PG alone did not reduce levels of exchangeable Al^{3+} in layers below 0.4 m compared with the control treatment. The SO_4^{2-}-S concentration was higher

throughout the soil profile when LPG was applied; in the treatments with L or PG alone, the SO_4^{2-}-S concentrations were similar and higher than that in the control treatment (Fig. 2F). SOC, P, Fe, Mn, and Zn contents in the topsoil (0.0–0.2 m) were significantly altered ($p < 0.01$; Table S3) by soil amendments (Fig. 3). In general, L-amended soils (regardless of PG addition) provided the highest levels of SOC (Fig. 3A) and P (Fig. 3B) and the lowest concentration of Fe, Mn, Cu and Zn (Fig. 3).

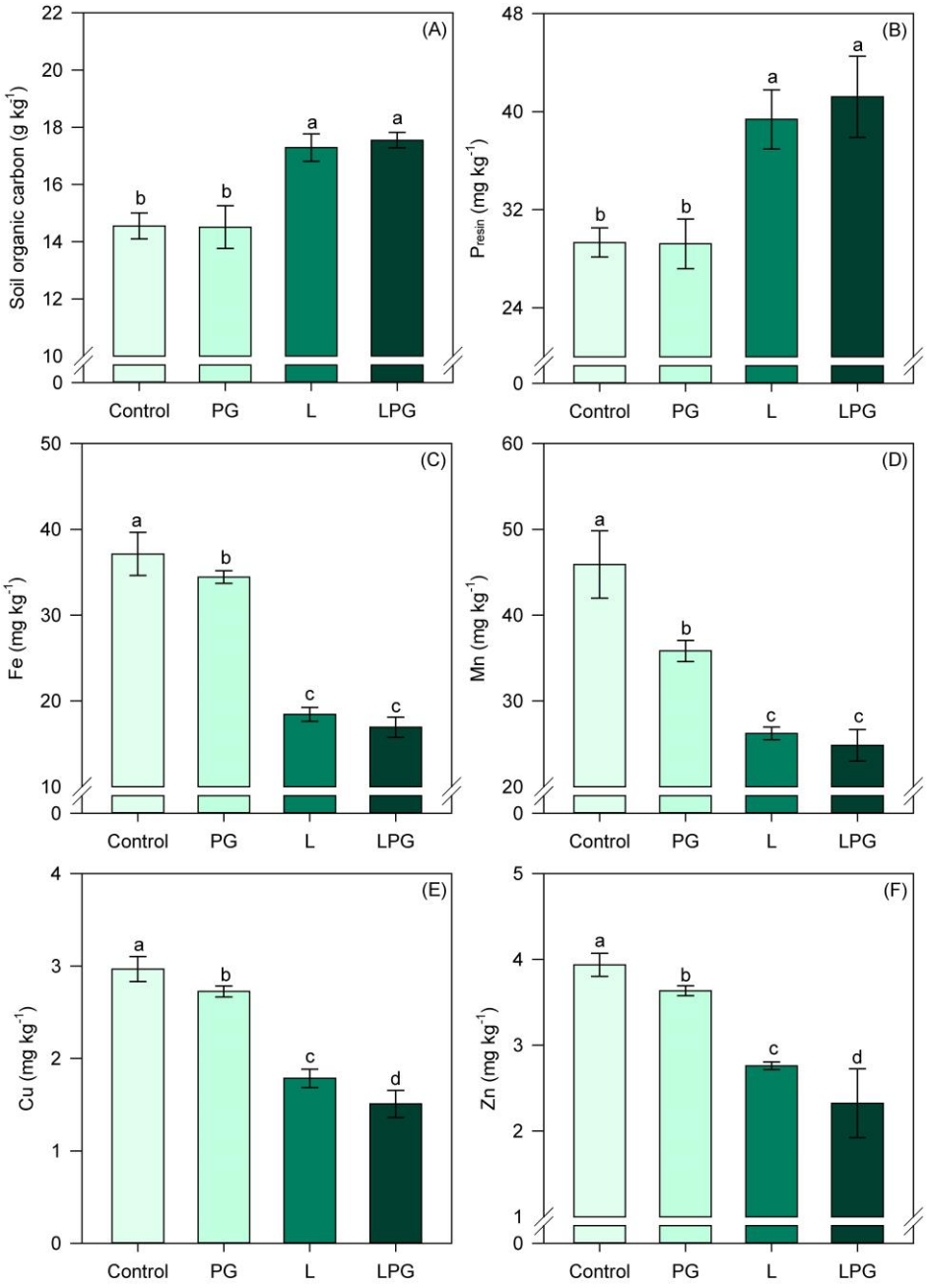


Figure 3. Changes in soil organic matter (SOC), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) at 0.0–0.2 m depth as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters for each soil depth indicate significant differences between

treatments by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

Maize root development composed by dry matter production and distribution, changed ($p < 0.01$; Table S4) by the long-term surface application of soil amendments (Fig. 4). In both growing seasons, root dry matter increased in L-amended soil, but combining L to PG (LPG treatments), the effects were enhanced, especially at deeper layers (below 0.4 m depth) (Fig. 4A and C). between treatments became more evident as the soil depth increased. Regardless of growing season, the root system distribution in the soil profile revealed that the density of roots was highest at 0.0–0.2 m soil layer in the control and PG treatments, with a lower proportion of roots in layers deeper than 0.2 m. In L-amended and, in particular, LPG-amended soil, the root distribution was more uniform throughout the soil profile (Fig. 4B and D). Treatment with PG alone improved root development and distribution compared with the control treatment, but the effects of PG alone were smaller than those of the L and LPG treatments. LPG-treatment provided the highest distribution of maize roots in the deepest soil depth.

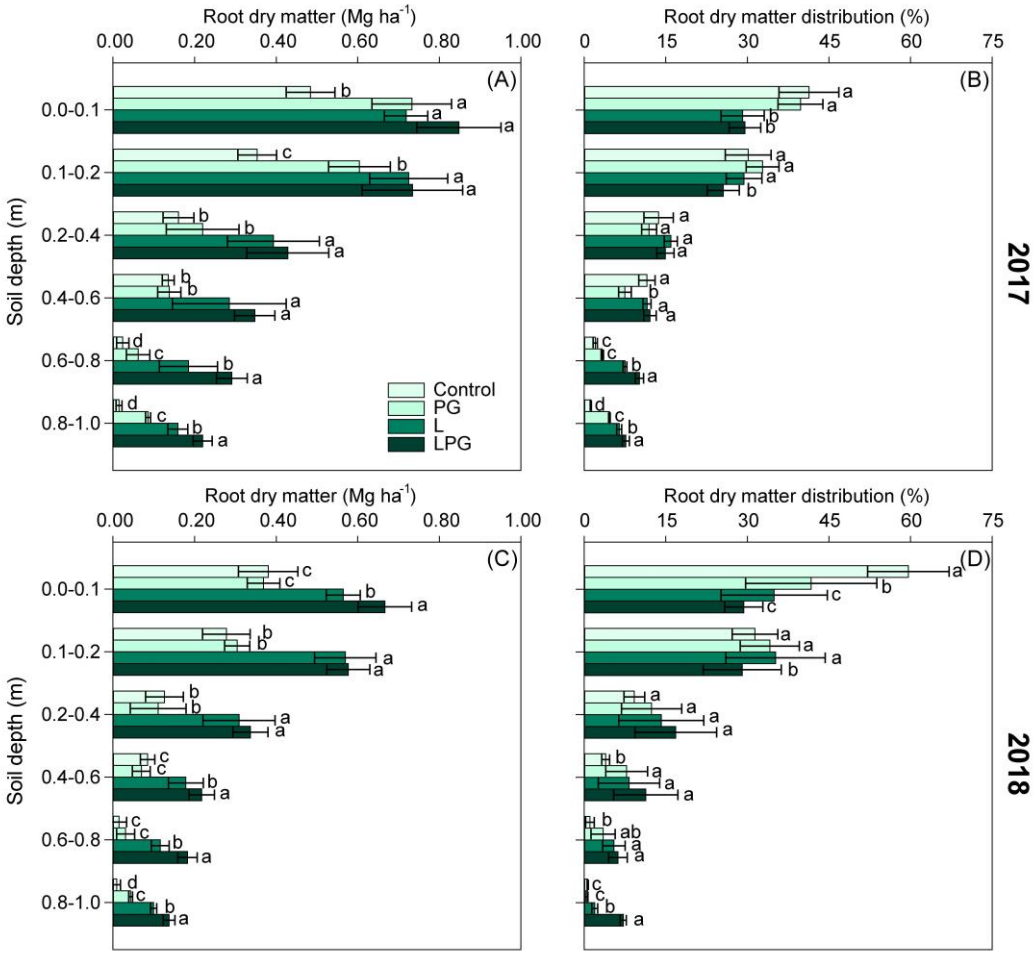


Figure 4. Root dry matter (A) and root dry matter distribution (B) in the soil profile as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters for each soil depth indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

2.3.3 Plant nutrition

Leaf macronutrient concentrations increased ($p < 0.05$; Table S6) in response to L and, in particular, LPG in both growing seasons (Table 1). With the exception of K which increased only in 2018, higher leaf concentration of N, P, Ca, Mg, and S occurred in both growing seasons in these treatments compared with the control and PG treatments. By contrast, the concentrations of Fe, Mn and Zn reduced in treatments L-based (L and LPG), as occurred according to the soil chemical analysis (Fig. 3). Among the micronutrients, leaf Cu concentration presented the smallest variation among micronutrients, showing changes only in 2018, where the control treatment provided higher concentrations than the other treatments.

Table 1. Influence of surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) on nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu and Zn) concentrations in the leaves of maize cultivated in two growing seasons in a long-term no-till system.

Leaf nutrients	units	Control		PG		L		LPG	
		2017	2018	2017	2018	2017	2018	2017	2018
N	g kg^{-1}	27.9 b	22.9 c	30.9 b	25.1 c	38.6 a	29.4 b	39.9 a	36.8 a
P	g kg^{-1}	2.21 b	1.81 b	2.32 ab	1.99 ab	2.33 ab	2.01 ab	2.41 a	2.21 a
K	g kg^{-1}	20.6 a	16.1 b	20.1 a	17.7 ab	20.8 a	18.3 ab	21.8 a	20.2 a
Ca	g kg^{-1}	1.81 c	2.13 b	4.18 a	2.34 ab	2.93 b	2.62 ab	3.78 ab	2.77 a
Mg	g kg^{-1}	2.20 b	1.81 c	2.03 b	1.99 c	5.30 a	4.39 b	5.88 a	5.63 a
S	g kg^{-1}	1.32 d	1.08 b	2.11 c	1.19 b	3.07 b	2.82 a	3.96 a	3.01 a
Fe	mg kg^{-1}	276 a	226 a	235 a	249 a	149 b	157 b	222 ab	164 b
Mn	mg kg^{-1}	30.6 a	25.1 a	23.8 b	27.6 a	21.5 b	19.6 b	21.9 b	19.4 b
Cu	mg kg^{-1}	13.7 a	11.7 ab	11.5 a	12.9 a	12.1 a	9.82 b	13.2 a	13.0 a
Zn	mg kg^{-1}	78.4 a	64.3 a	82.1 a	70.6 a	42.4 b	39.4 b	46.5 b	35.9 b

Different lower-case letters on the lines indicate significant differences between treatments by Student's t-test at $p \leq 0.05$. †Low significant difference (LSD), ‡coefficient of variation (CV). Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), cupper (Cu) and zinc (Zn).

2.3.4 Photosynthetic pigments and gas exchange measurements

Regardless of growing season, the application of L and particularly LPG increased (p <0.01; Table S7) the concentrations of chlorophylls (*a*, *b* and total) and carotenoids compared with the control and PG treatments (Fig. 5).

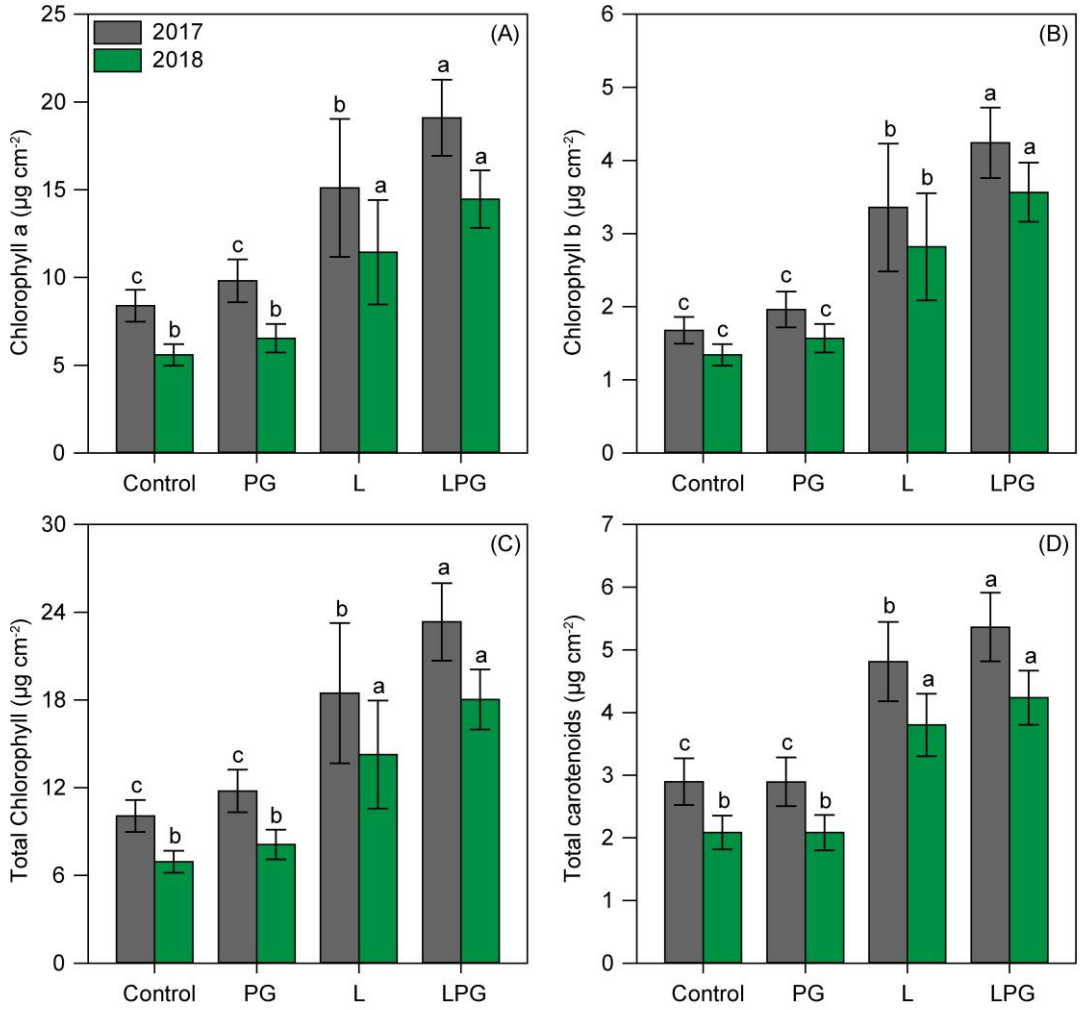


Figure 5. Chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and carotenoids (D) contents in maize leaves as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 4).

On average, considering all photosynthetic pigments, LPG-amended soil provided maize plants with 137% more pigments than the control treatment, followed by 90% more in the treatment with L and 15% with PG. Leaf gas exchange of maize in both growing seasons improved by applying LPG, followed by L (Fig. 6) compared with control and PG. In LPG-amended soil, maize plants presented the largest *A* rate compared with control treatments (LPG = 70%; L = 57%), as well as *gs* rates increased

by 478% in LPG, by 380% in L, and by 76% in PG treatments (Fig. 6A and B). Lowest rates of *ic* (Fig. 6C) and *E* (Fig. S1) also occurred in these treatments (L and LPG), although differences did not occur between them. As a consequence of improvements in *A* and *E* rates, higher WUE occurred maize cultivated in L (250%) and LPG-amended soils (278%), both compared with control (Fig. 6D).

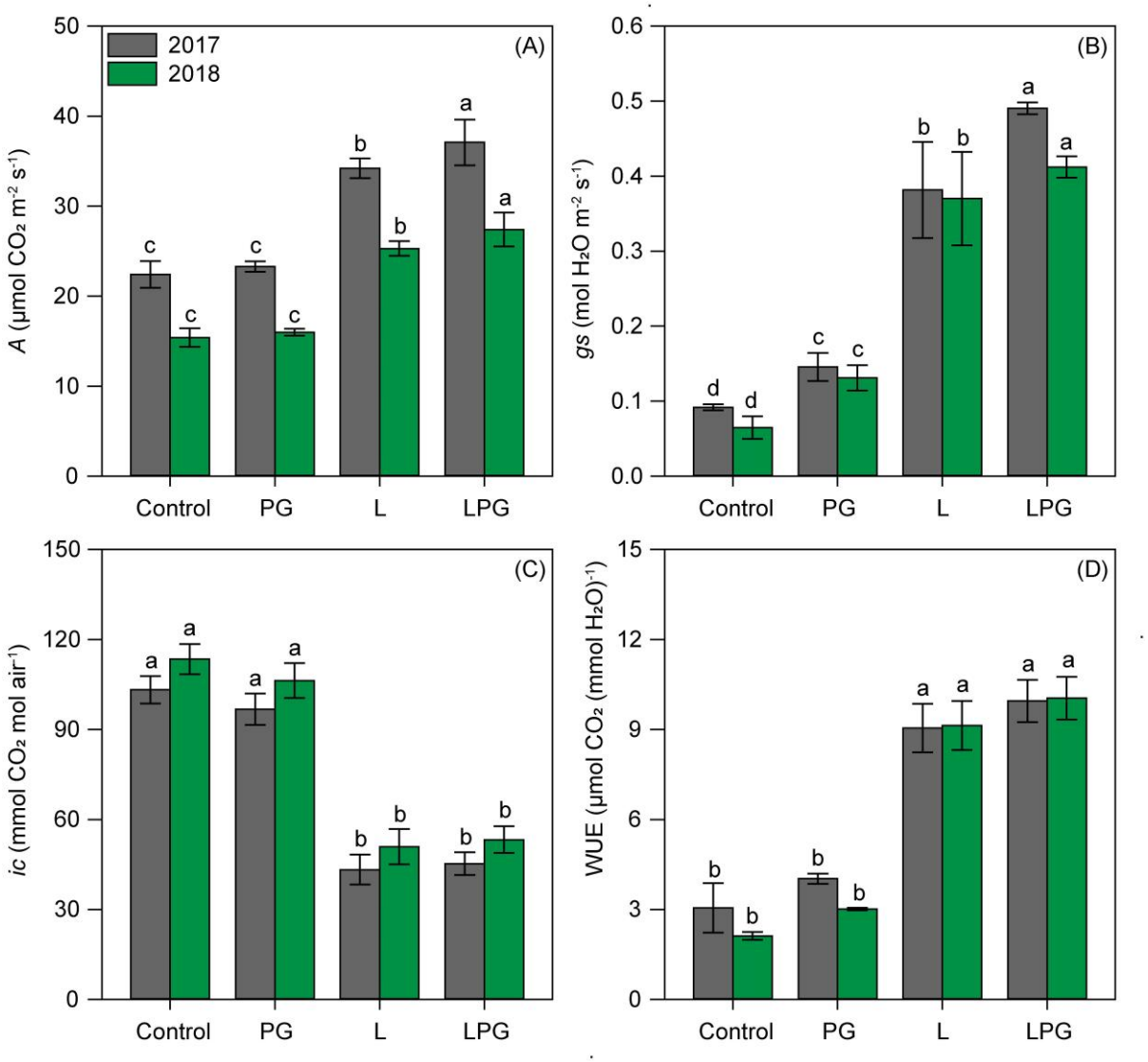


Figure 6. Net photosynthesis rate–*A* (A), stomatal conductance–*gs* (B), internal CO_2 concentration–*ic* (C), and water use efficiency–WUE (D) in maize leaves as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

2.3.5 Carbon metabolism

The increased pigment concentrations in response to soil amendments were positively reflected in Rubisco activity (Fig. 7A). Rubisco activity was highest ($p < 0.01$; Table S7) in maize cultivated in LPG-amended soil (67% higher than control), followed by the L treatment (55% higher than control).Fig. 7 The sucrose concentration was highest ($p < 0.01$; Table S7) in the control treatment in both growing seasons (Fig. 7B). In general, sucrose concentration in maize leaves decreased by 24% from control to the L and LPG treatments. The pattern of Susy activity was similar to that of Rubisco activity; Susy activity was highest ($p < 0.01$; Table S7) in maize plants in the LPG-amended soil (120% higher than control), followed by the application of L alone (90% higher than control) (Fig. 7C).

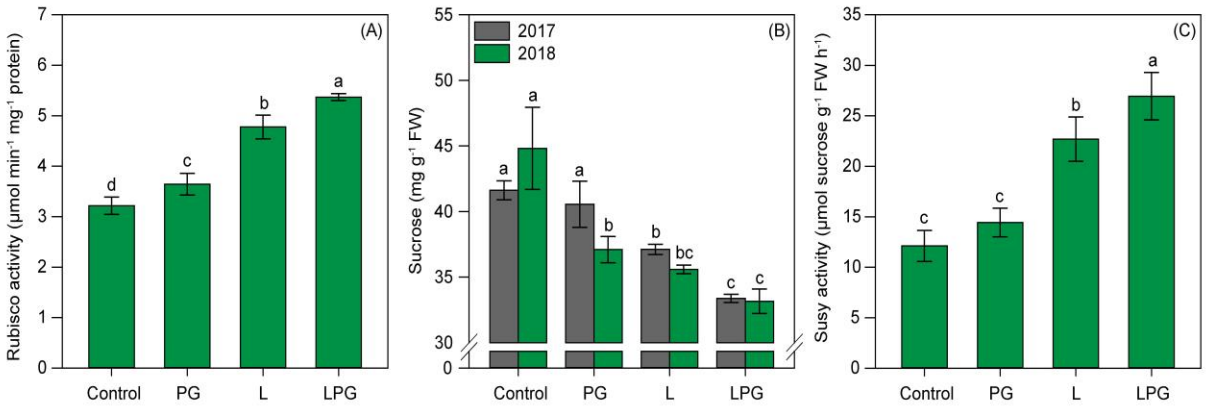


Figure 7. Rubisco activity (A), sucrose concentration (B), and Susy activity (C) in maize leaves as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

2.3.6 Lipid peroxidation and antioxidant metabolism

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations in maize leaves were highest ($p < 0.01$; Table S7) in the control treatment (unamended soil) (Fig. 8A and B). Regardless of growing season, the concentrations of H_2O_2 and MDA were reduced ($p < 0.01$; Table S7) by all of the soil amendments compared with the control; however, these reductions were greatest in the LPG treatment (H_2O_2 = 66%; MDA = 54%), followed by the treatments with L (H_2O_2 = 53%; MDA = 13.7%) and PG (H_2O_2 = 24.6%; MDA = 6.4%) alone.

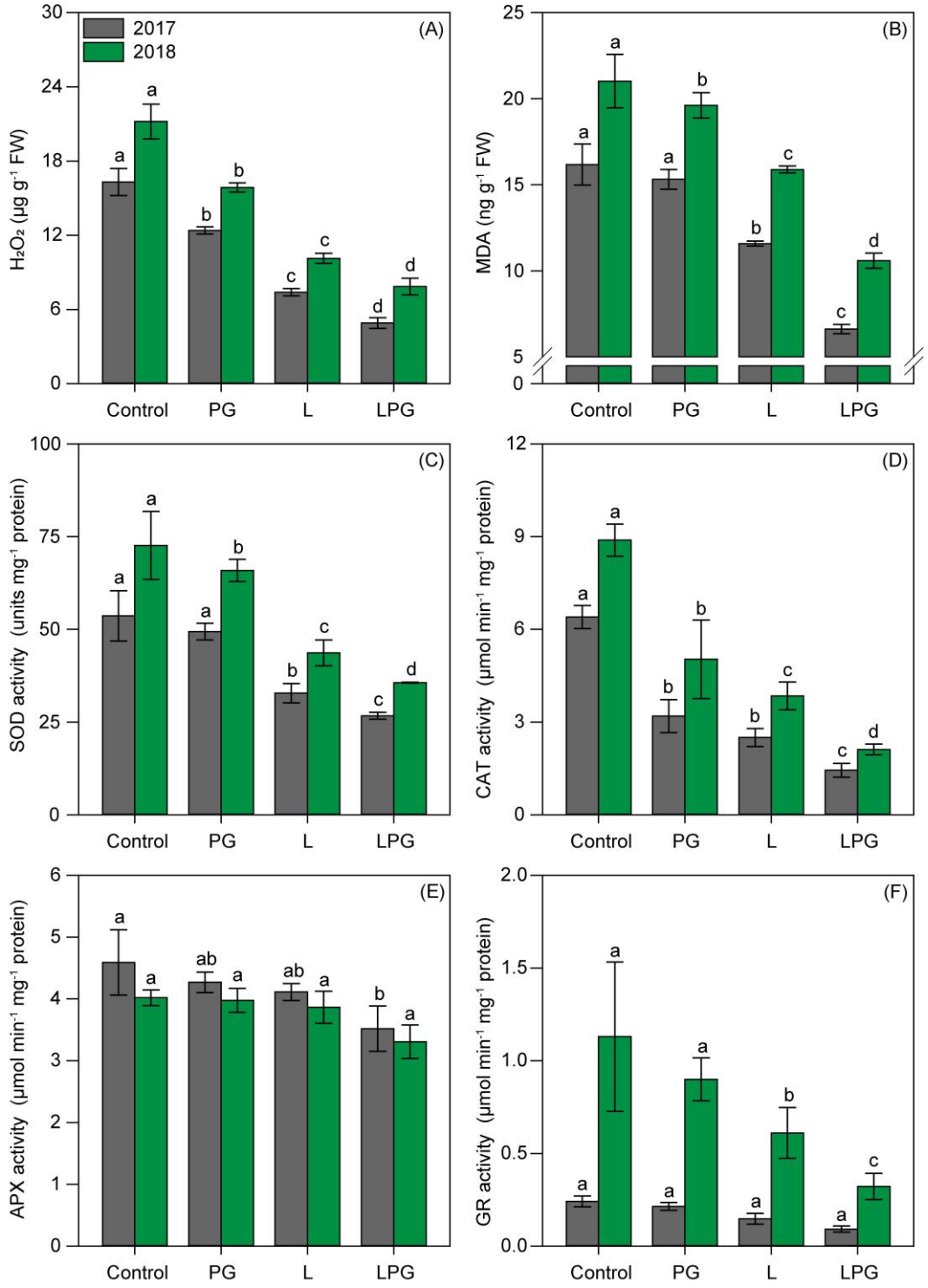


Figure 8. Oxidative stress [hydrogen peroxide (H_2O_2) (A) and malondialdehyde (MDA) (B) concentrations)] and antioxidant enzyme activities [superoxide dismutase–SOD (C), catalase–CAT (D), ascorbate peroxidase–APX (E), and glutathione reductase–GR (F)] in maize leaves as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

Antioxidant enzyme activities were highest ($p < 0.01$; Table S7) in the leaves of maize in the control treatment or the treatment with PG alone (Fig. 8C–F) in both growing seasons. On the other hand, enzymatic activity, *i.e.*, SOD (Fig. 8C), CAT (Fig. 8D), APX (Fig. 8E), and GR (Fig. 8F) was lower ($p < 0.01$; Table S7) in L-amended soil, especially in the LPG treatment. In general, considering both growing seasons, the antioxidant enzyme activities in maize cultivated in LPG-amended soil reduced by 51% for SOD, by 77% for CAT, by 21% for APX, and by 70% for GR, when compared with control treatment.

2.3.7 Maize shoot dry matter production and grain yield

Considering the average between the two growing seasons, shoot dry matter (SDM) and grain yield (GY) were highest ($p < 0.01$; Table S7) in the LPG treatment (SDM = 92%; GY = 260%), compared with control, followed by the L (SDM = 70%; GY = 200%), and PG (SDM = 1.35%; GY = 31%) (Fig. 9A and B). A clear trend of LPG > L > PG > control was also observed for the development of maize plants at 50 days after emergence (DAE) and ears obtained at harvest in the second growing season (Fig. 9C), although some similarities were found between the L and LPG treatments and between the control and PG treatments.

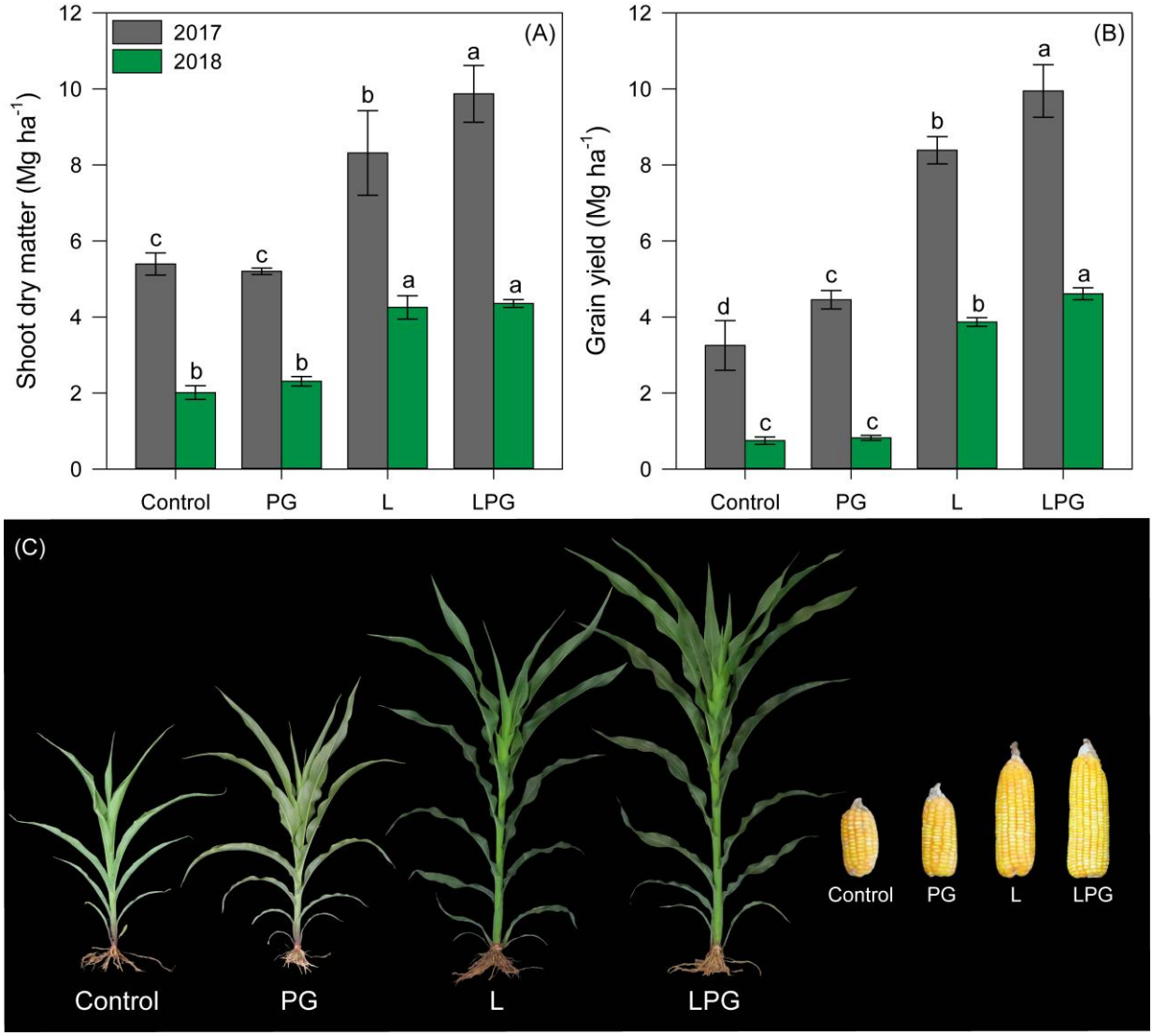


Figure 9. Shoot dry matter (A) and grain yield (B) of maize as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 4). Effect of soil amendments on maize development (2nd growing season = 2018) at 50 days after sowing and ear development at harvest (C).

2.3.8 Redundancy and correlation analyses of soil and maize plant measurements

To elucidate the effects of soil fertility (considering the average of soil attributes at the 0.0–1.0m depth) on crop nutrition and crop physiology (average of the 2 growing seasons), three RDAs were performed (Fig 10A-C). The first RDA contemplates the relationship between soil fertility and crop nutrition, where axis 1 indicated that the soil factors explained 93.1% of the variation in nutrient concentration in maize leaves (Fig.

10A). PERMANOVA analysis segregated the treatments into the following three groups: group 1 represented by the control, group 2 composed by PG, and group 3 composed by L and LPG treatments. In addition, according to Monte Carlo permutation, the main soil factors ($p < 0.05$) responsible for all variation in crop nutrition were the soil concentrations of Ca^{2+}, Mg^{2+}, P_{resin}, and SO_4^{2-}- S. Considering the role of soil fertility on crop physiology, PERMANOVA analysis segregated the treatments in four distinct groups, each represented by a treatment, with axis 1 indicating that soil fertility explained 93.8% of all variation in crop physiology (Fig. 10B) . The main soil factors ($p < 0.05$) responsible for these variations were the concentrations of Ca^{2+}, Mg^{2+}, P_{resin} and Mn. On the other hand, linking crop nutrition with maize physiology by RDA, axis 1 indicated that the crop nutrition was responsible for 91.5% of all variation in plant physiology (Fig. 10C). In addition, PERMANOVA analysis segregated the treatments in two groups. Group 1 was composed by control and PG, whereas group 2 was composed by L and LPG treatments. Among the nutrients, Monte Carlo permutation indicated that the leaf concentrations of N, P, Mg and Mn were the main responsible for changing ($p < 0.05$) the patterns of carbon and antioxidant metabolism in the maize. In general, Mn (soil or plant) has always been associated with an increase in oxidative stress, whereas the macronutrients (especially P and Mg) were associated with improved carbon metabolism.

Overall, correlation analysis of soil fertility and maize nutritional, physiological and productive attributes demonstrated that improvements in soil chemical properties enhanced the development and distribution of roots in the soil profile, resulting in higher concentration of nutrients in maize leaves, as well as improved the carbon fixation, lower oxidative stress and greater shoot dry matter and grain yield (Fig. 10D). Increased pH and the corresponding effects on soil properties (*i.e.*, increased SOC and exchangeable cations and low Al^{3+} toxicity up to 1.0 m depth) were positively correlated with root growth, leaf macronutrient concentration, pigment concentrations, gas exchange parameters, Rubisco and Susy activities, and shoot and grain production. Additionally, increased pH was negatively correlated with leaf and soil micronutrient concentrations, lipid peroxidation and the activities of SOD, CAT, APX, and GR.

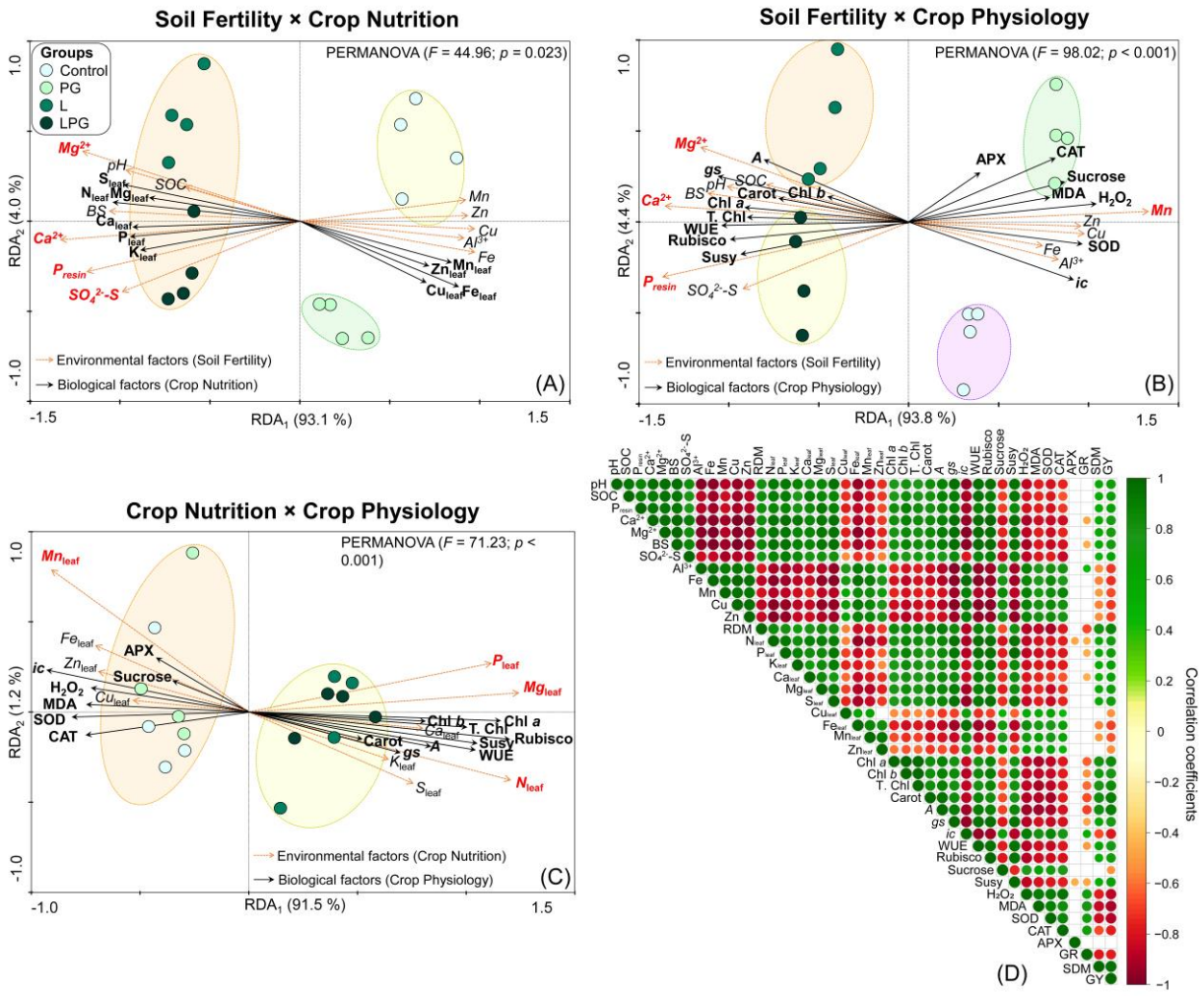


Figure 10. Redundancy analysis triplot (RDA) showing the relationship between the soil fertility × crop nutrition (A), soil fertility × crop physiology (B), and crop nutrition × crop physiology (C). The canonical axes are labeled with percentage of total variance explained (%). The arrows indicate correlations between factors. The significance of these correlations was evaluated by a Monte Carlo permutation test with 999 permutations and the significant soil properties are indicated by red color ($p \leq 0.05$). The color dashed lines indicate significant clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). Heatmap showing the correlation coefficients (Pearson) among the soil fertility, root growth, crop nutrition, crop physiology, and agronomic parameters of maize plants (D). Only significant correlations at $p \leq 0.05$ are shown. Soil organic matter (SOC), base saturation (BS), sulfate (SO_4^{2-}-S), root dry matter (RDM), chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), total chlorophyll (T. Chl), carotenoids (Carot), Net photosynthesis rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*ic*), water use efficiency (WUE), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), shoot dry matter (SDM) and grain yield (GY).

2.4 DISCUSSION

2.4.1 Climatic conditions

In both growing seasons, the amounts of rainfall received during maize cropping were much lower than the range (400 to 600 mm) considered sufficient for crop development (Fancelli, 2015), particularly in the second growing season (105 mm less than 2017), resulting in a severely negative hydric balance during maize development (Fig. 1). In both growing seasons, the hydric conditions for second-crop maize were inappropriate and negatively influenced crop development and yield. According to Gupta et al. (2020), drought is responsible for more annual losses of crop grain yield than all plant pathogens combined. Climatic anomalies related to longer periods of rain scarcity are becoming increasingly common in tropical regions (Cunningham, 2020; Heinemann et al., 2021), limiting food production. Tropical regions with weathered and acidic soils present low nutrient availability and high Al^{3+} levels, leading to restricted root growth and lower uptake of water and nutrients, especially under lower water supply (Ritchey et al., 1982; Joris et al., 2013).

2.4.2 Changes in soil chemical properties, root development and plant nutrition

Remarkable effects of the soil amendments, primarily L and LPG, were observed for most soil chemical properties, including higher soil pH, Ca^{2+}, Mg^{2+} and SO_4^{2-}-S levels and lower levels of exchangeable Al^{3+} throughout the soil profile (Fig. 1). As a result, there were significant correlations among soil pH, macronutrient availability and BS (Fig. 10B). Additionally, in the 0.0–0.2 m layer, SOC content and P_{resin} concentration increased by the soil amendments application, although the increase in pH resulted in lower availability of cationic micronutrients (Fig. 3 and 8B). When PG was applied alone, soil attributes were slightly improved compared with the control treatment, but the effects were much smaller than those obtained by liming, regardless of the soil layer. However, when PG was applied in combination with L (LPG), the results were oftentimes greater than those obtained with L alone, especially in deeper soil layers. Unlike L, PG cannot correct soil acidity (Zoca and Penn, 2017), so its direct effects are linked to increased availability of Ca^{2+} and SO_4^{2-}-S and reduced Al^{3+} content, mainly in deep layers (Costa et al., 2018; Crusciol et al., 2019; Bossolani

et al., 2021b). Since most soil properties co-vary with pH (Lammel et al., 2018), PG is an important complementary amendment but not a substitute for L (Bossolani et al., 2020a).

Changes in soil nutrient concentrations can alter root development in the soil profile (Rellán-Álvarez et al., 2016). Positive correlations with soil fertility occurred for root dry matter (Fig. 10B). During plant development, the architecture of the root system undergoes morphological changes depending on the availability of water and nutrients in order to improve the acquisition of environmental resources (Carmeis Filho et al., 2017; Bossolani et al., 2021b). In tropical acidic soils, low Ca^{2+} availability and high Mn and Al^{3+} availability in deeper layers are the main causes of restricted root development. Calcium plays a fundamental role in root growth as a component of the hormonal peptides responsible for cell elongation (Ritchey et al., 1995), and when it is absent in deeper layers, roots become superficial, increasing the susceptibility of plants to drought stress and limiting nutrient uptake (Crusciol et al., 2019; Bossolani et al., 2021b). The root distribution in the soil profile can be traced to mechanisms of cell division, elongation, and differentiation mediated by nutrient availability (Rellán-Álvarez et al., 2016). Longer and deeper roots can efficiently take up water from deep layers (Gupta et al., 2020).

Nutrient uptake by maize increased in L- and LPG-amended soils (Table S5). In general, the leaf concentration of all macronutrients was positively influenced by L (regardless of PG addition). However, the leaf concentration of cationic micronutrients decreased in these treatments in response to their reduced availability in soil (Fig. 1), as supported by correlation analysis (Fig. 10D). In addition, our RDA analysis regarding the interactions between soil fertility and crop nutrition revealed the primary effects higher soil concentrations of Ca^{2+}, Mg^{2+}, P_{resin} and SO_4^{2-}-S on improving the crop nutrition (Fig. 10A). Thus, combining higher root development with increased soil nutrient concentrations, results on well-nourished plants that are able to withstand periods of low rainfall (Carmeis Filho et al., 2017; Bossolani et al., 2021b). In addition, according to Malavolta (1997), the macronutrient contents in maize were within the optimum range for full development only in soils amended with L or LPG, in both growing seasons.

2.4.3 Photosynthetic parameters and carbon metabolism response

Amendment with L or LPG (Fig. 1 and Fig. 3) enabled greater root development and improved root distribution throughout the soil profile (Fig. 2), as well as plant nutrition (Table S5). Under low water availability like that observed in this study, plants seek to adapt to the soil moisture gradient and, driven by nutrient availability, change their root architecture to enhance their ability to take up water and nutrients (Rellán-Álvarez et al., 2016). These changes are reflected in greater synthesis of chlorophylls and carotenoids (Fig. 5). Chlorophyll is an important part of the Calvin cycle and is responsible for harvesting sunlight during plant photosynthesis (Croft et al., 2017; Busch, 2020). Carotenoids are responsible for adapting plastids to stress conditions, light and energy dissipation to avoid excessive production of reactive oxygen species (ROS) (Gómez et al., 2019).

Correlation analysis suggested that the increase in photosynthetic pigment concentrations was related to changes in leaf nutritional status, gas exchange parameters and photosynthetic potential (Fig. 10D). Rates of *A*, *gs* and WUE were also increased by applying L alone or LPG (Fig. 6A, B and D). In addition, the increases in these parameters in association with the decreases in *ic* (Fig. 6C) and *E* (Fig. 5) imply greater productive capacity of plants under stress conditions (Reis et al., 2018). The regulation of stomatal conductance under conditions of low hydric availability is extremely important for increased WUE (Gupta et al., 2020). According to these authors, stomatal closure is the first defense against water loss. Adequate plant nutrition (*e.g.*, K, Ca, and Mg) are essential for the mechanisms that modulate this response (Brodribb and McAdam, 2017; Gong et al., 2020). According to our RDAs linking soil fertility (Fig. 10B) and crop nutrition (Fig. 10C) with crop physiology, *gs* values were strongly associated with the soil concentrations of exchangeable bases (Ca^{2+}, Mg^{2+} and BS) and the leaf concentrations of K and Ca, reinforcing the role of these nutrients in improving the use of water under hydric restrictions, as occurred in our study. Additionally, greater availability of nutrients in the soil, especially in deeper layers, increases root growth and the use of resources that enhance resilience to low water availability (Rellán-Álvarez et al., 2016).

The combination of these factors may imply greater carbon fixation by plants (Reis et al., 2018). The key enzyme involved in carbon fixation that drives the assimilation of CO_2 is Rubisco (Busch, 2020). In this study, Rubisco activity was higher

in the leaves of maize in soils amended with L and, in particular, LPG (Fig. 7A). Susy activity was also increased in these treatments (Fig. 7C), although the levels of sucrose decreased (Fig. 7B). The response pathways of plants to environmental stress also include changes in the production and mobilization of metabolites (Bailey-Serres et al., 2019). Each Rubisco-mediated carboxylation reaction gives rise to triose phosphates that are exported and become substrates for the synthesis of most of the other organic compounds that make up the plant (Stein and Granot, 2019). Sucrose synthase (Susy) plays a key role in sugar metabolism and can reversibly cleave sucrose into fructose and glucose. These by-products can enter several metabolic pathways to provide energy and carbon skeletons for plant metabolism (Stein and Granot, 2019). The increases in Rubisco and Susy activity and low sucrose concentrations in leaves suggest enhancement of production and partitioning of sugars from source to sink tissues (Farhat et al., 2016).

RDA enabled the identification of the most important soil and nutritional factors responsible for increasing carbon metabolism (Fig. 10B and C), that were also supported by correlation analysis (Fig. 10D). Both RDAs presented a similar pattern correlating the soil and nutritional factors on carbon metabolism, however, the segregation of treatments was assembled differently. Soil fertility was more sensitive in clustering the treatments, segregating each treatment in a group; on the other hand, crop nutrition separated the treatments into only two groups: control and PG, and L and LPG treatments. Possibly, the segregation of treatments occurred differently considering soil fertility and crop nutrition due to the strong role of soil chemistry in modulating the soil nutrients availability, and the root growth, which directly contributes to the acquisition of water for the full functioning of photosynthesis (Rellán-Álvarez et al., 2016; Busch, 2020; Gong et al., 2020). Additionally, although the concentration of nutrients is an important indicator of the nutritional status of plants, it is worth noting that the leaf concentration of nutrients does not reflect the amount of nutrients that accumulate in the plant tissues. The foliar concentration of nutrients can be subject to the concentration and dilution processes, depending on the biomass production by plants (Maia, 2012). For this reason, the soil chemistry may have been more sensitive to changes that occurred in carbon metabolism. In general, soil and leaf P, Ca^{2+}, and Mg^{2+} were the main nutrients responsible for improving the dynamics of Rubisco and Susy activities in maize plants, whereas Mn was mainly responsible for reductions in these activities, as observed in the control and PG treatments. Phosphorus is an

important supplier of energy for photosynthetic mechanisms and plant metabolism. Mg^{2+} is the most abundant divalent cation in the plant cytosol and participates in several aspects of photosynthesis, such as the composition of the chlorophyll molecule, activation of Rubisco in the carboxylation process and the partitioning of photoassimilates by plant tissues (Ceylan et al., 2016). Calcium also plays an important role in multiple photosynthetic pathways (Wang et al., 2019). This nutrient affects gas exchange related to photosynthesis by regulating stomatal movement (Song et al., 2014). Several photosynthetic proteins are regulated directly or indirectly by Ca (Wang et al., 2019). In addition, soil Ca plays an important role in signaling and root growth, increasing the capacity of plants to uptake water and other nutrients (Ritchey et al., 1995).

In acidic and low-fertility soils, Mn is generally abundant and limits plant growth (Roth and Pavan, 1991; Bossolani et al., 2020b). Mn can replace Mg in the Rubisco activation process and increases the affinity of the enzyme for oxygen by approximately 20-fold compared with Mg^{2+}, thus increasing photorespiration by plants (Kitao et al., 1997). In addition, high concentrations of metallic nutrients such as Mn and Al^{3+} can reduce the WUE of plants (Reis et al., 2018), compromising the crops in periods of low water availability. Several authors have also associated these elements with reduced root development (Roth and Pavan, 1991; Ritchey et al., 1995; Caires et al., 2011; Costa and Crusciol, 2016).

2.4.4 Lipid peroxidation and antioxidant metabolism response

Oxidative stress in maize plants was strongly reduced by the application of L and LPG (Fig. 8A and B), as evidenced by the reductions in the concentrations of H_2O_2 and MDA in these treatments. In addition to its role in cell defense signaling (Cuypers et al., 2010), H_2O_2 is one of the main forms of ROS responsible for lipid peroxidation (Loix et al., 2018).

The patterns of activity of antioxidant system enzymes (SOD, CAT, APX and GR) (Fig. 8C-F) were similar to those for lipid peroxidation, indicating that the low activity of these enzymes in maize established in L- and LPG-amended soils is the result of efficient catalysis of their substrates (ROS) (Silva et al., 2020). Lipid peroxidation can also be caused by other ROS [*e.g.*, singlet oxygen ($^1O_2^-$) and hydroxyl radicals (OH^-)] (Farmer and Mueller, 2013). The first line of defense in antioxidant

metabolism is the dismutation of $^1O_2^-$ into H_2O_2 and H_2O by the enzyme SOD, followed by the breakdown of H_2O_2 into H_2O and O_2 by CAT, APX, and GR (Noctor, 2002). In this process of ROS scavenging, enzymes are also consumed, resulting in reduced activity when measured in plant tissue (Noctor, 2002; Farmer and Mueller, 2013).

The increased oxidative stress in maize plants in the control and PG treatments is primarily attributable to low soil fertility and plant nutrition, as supported by RDA and correlation analysis (Fig. 10A-D). Plants grown under low nutrient availability, mainly P, Ca^{2+}, and Mg^{2+}, and under high concentrations of toxic elements, such as Mn and Al^{3+}, are more likely to reduce pigment levels (Fig. 5) and photosynthetic activity (Fig. 6 and 5) (Liu et al., 2008, 2015; Gong et al., 2020), in addition to accumulating sugars in source tissues (Fig. 7B) (Stein and Granot, 2019). All of these factors are positively correlated with increased oxidative stress (Fig. 10B) (Gong et al., 2020). The gradual loss of photosynthetic capacity results in electron accumulation in photosystems I and II, generating greater amounts of ROS and lipid peroxidation (Croft et al., 2017). The accumulation of sucrose in photosynthetically active leaves interferes with the production of ROS and increases oxidative stress in plants, especially for plants growing under soil Mg^{2+} deficiency (Cakmak and Kirkby, 2008). Calcium deficiency induces low stomatal conductance (Fig. 6B and 8B) and, consequently, higher water loss via stomata, which can increase oxidative stress in plants (Gupta et al., 2020), particularly under low hydric availability, which is common during maize cropping. In summary, the mitigation of soil acidification, reduced toxicity of Mn and Al^{3+}, and increased availability of nutrients such as P, Ca^{2+} and Mg^{2+} provided by liming (L), especially when combined with PG (LPG), can reduce oxidative stress in plants.

2.4.5 Maize shoot dry matter production and grain yield

This study revealed positive effects of the soil amendments on root development, plant carbon and antioxidant metabolism, shoot dry matter production and grain yield (Fig. 9). In addition, during maize development, more turgid and vigorous plants were obtained, even under low pluvial precipitation (Fig. 1). The long-term application of L and LPG improved soil fertility and root development, increasing the water and nutrients uptake by plants (Table 1). In addition, increased photosynthesis and better regulation of oxidative stress led to higher maize grain yields under these soil amendments. Importantly, combining L and PG (LPG) provided better

results than amendment with L alone. Understanding how soil amendments changes tropical soils and shape plant responses under drought is of paramount importance for the development of more productive agricultural systems under continuous climate change (Gibbons et al., 2014; Gupta et al., 2020). Therefore, amendment of soils with LPG should be considered a viable and promising alternative to improve the yield capacity of acidic tropical soils managed under NT (Costa and Crusciol, 2016; Crusciol et al., 2019; Bossolani et al., 2020a, 2020b, 2021b).

2.5 CONCLUSIONS

After two years of the last surface reapplication of soil amendments (4 reapplications of lime and/or phosphogypsum in 16 years of experiment), liming, especially combined with phosphogypsum, increased soil pH, P, Ca^{2+}, Mg^{2+} and SO_4^{2-}-S and reduced the concentrations of Al^{3+}, Fe, Mn, Cu and Zn beyond the depth where lime was applied. The improvement on soil fertility brought about by soil amendments, led to an increase on root growth of maize, which in turn, increased water and nutrient uptake by plants. As a result of this chain effect, maize plants grown under field drought conditions, improved their antioxidant system, photosynthetic pigment concentrations and, consequently, the carbon metabolism, including Rubisco and Susy activities, and sucrose production and partitioning. This study has not only confirmed that phosphogypsum potentiates the effects of lime on improving soil fertility, but has also highlighted that the increase in macronutrient concentrations (in soil and plants), especially P, Ca and Mg, and reducing Mn concentrations, has a fundamental role in increasing antioxidant system and photosynthetic metabolism, benefits that eventually were reflected in the increase of the biomass production and grain yield.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by São Paulo Research Foundation (FAPESP) (Grant 2018/11063-7 and 2019/12764-1) through financial support for scholarship to the first author and for analysis. In addition, the National Council for Scientific and Technological Development (CNPq) (Universal Research Project: 421637/2018-8)

provided financial support for analysis to the second author. Finally, the authors CACC, EFC, TJCA, JCC and ARR also would like to thank the CNPq for an award for excellence in research.

REFERENCES

Alexieva, V., Sergiev, I., Mapelli, S., and Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant. Cell Environ.* 24, 1337–1344. doi:https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Allen, R. G., Pereira, L. S., Raes, D., and Smith, M. (1998). Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. *Fao, Rome* 300, D05109.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., de Moraes Gonçalves, J. L., and Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorol. Zeitschrift* 22, 711–728. doi:https://doi.org/10.1127/0941-2948/2013/0507.

Anderson, M. J. (2005). Permutational multivariate analysis of variance. *Dep. Stat. Univ. Auckland, Auckl.* 26, 32–46.

AOAC (2016). Official methods of analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5.

Azevedo, Ra., Alas, R. M., Smith, R. J., and Lea, P. J. (1998). Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. *Physiol. Plant.* 104, 280–292. doi:https://doi.org/10.1034/j.1399-3054.1998.1040217.x.

Bailey-Serres, J., Parker, J. E., Ainsworth, E. A., Oldroyd, G. E. D., and Schroeder, J. I. (2019). Genetic strategies for improving crop yields. *Nature* 575, 109–118. doi:10.1038/s41586-019-1679-0.

Bardsley, C. E., and Lancaster, J. D. (1960). Determination of reserve sulfur and soluble sulfates in soils. *Soil Sci. Soc. Am. J.* 24, 265–268. doi:https://doi.org/10.2136/sssaj1960.03615995002400040015x.

Bian, M., Zhou, M., Sun, D., and Li, C. (2013). Molecular approaches unravel the mechanism of acid soil tolerance in plants. *Crop J.* 1, 91–104. doi:10.1016/j.cj.2013.08.002.

Bieleski, R. L., and Turner, N. A. (1966). Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Anal. Biochem.* 17, 278–293.

Bindraban, P. S., van der Velde, M., Ye, L., van den Berg, M., Materechera, S., Kiba, D. I., et al. (2012). Assessing the impact of soil degradation on food production. *Curr. Opin. Environ. Sustain.* 4, 478–488. doi:10.1016/j.cosust.2012.09.015.

Bossolani, J. W., Crusciol, C. A. C., Leite, M. F. A., Merloti, L. F., Moretti, L. G., Pascoaloto, I. M., et al. (2021a). Modulation of the soil microbiome by long-term

Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. *Soil Biol. Biochem.* 156. doi:10.1016/j.soilbio.2021.108188.

Bossolani, J. W., Crusciol, C. A. C., Merloti, L. F., Moretti, L. G., Costa, N. R., Tsai, S. M., et al. (2020a). Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. *Geoderma* 375, 114476. doi:10.1016/j.geoderma.2020.114476.

Bossolani, J. W., Crusciol, C. A. C., Portugal, J. R., Moretti, L. G., Garcia, A., Rodrigues, V. A., et al. (2021b). Long-term liming improves soil fertility and soybean root growth, reflecting improvements in leaf gas exchange and grain yield. *Eur. J. Agron.* 128, 126308. doi:10.1016/j.eja.2021.126308.

Bossolani, J. W., dos Santos, F. L., Meneghette, H. H. A., Sanches, I. R., Moretti, L. G., Parra, L. F., et al. (2020b). Soybean in Crop Rotation with Maize and Palisade Grass Intercropping Enhances the Long-term Effects of Surface Liming in No-till System. *J. Soil Sci. Plant Nutr.* 20, 1–12. doi:10.1007/s42729-020-00347-2.

Bossolani, J. W., Lazarini, E., Santos, F. L., Sanches, I. R., Meneghette, H. H. A., Parra, L. F., et al. (2018). Surface Reapplication of Lime and Gypsum on Maize Cultivated Sole and Intercropped with Urochloa. *Commun. Soil Sci. Plant Anal.* 49, 1855–1868. doi:10.1080/00103624.2018.1475565.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi:https://doi.org/10.1006/abio.1976.9999.

Brodribb, T. J., and McAdam, S. A. M. (2017). Evolution of the stomatal regulation of plant water content. *Plant Physiol.* 174, 639–649. doi:10.1104/pp.17.00078.

Busch, F. A. (2020). Photorespiration in the context of Rubisco biochemistry, CO2 diffusion and metabolism. *Plant J.* 101, 919–939. doi:10.1111/tpj.14674.

Caires, E. F., and Guimarães, A. M. (2018). A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. *Agron. J.* 110, 1987–1995. doi:10.2134/agronj2017.11.0642.

Caires, E. F., Joris, H. A. W., and Churka, S. (2011). Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. *Soil Use Manag.* 27, 45–53. doi:10.1111/j.1475-2743.2010.00310.x.

Cakmak, I., and Kirkby, E. A. (2008). Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol. Plant.* 133, 692–704. doi:10.1111/j.1399-3054.2007.01042.x.

Cantarella, H., RAIJ, B. van, and Camargo, C. E. O. (1997). Adubação de cereais. *RAIJ, B. van al., eds. Recom. adubação e calagem para o Estado São Paulo* 2, 43–50.

Carmeis-Filho, A. C. A., Crusciol, C. A. C., and Castilhos, A. M. (2017). Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. *J. Agric. Sci.* 155, 1093–1112. doi:10.1017/S0021859617000235.

Carmeis Filho, A. C. A., Crusciol, C. A. C., and Castilhos, A. M. (2017). Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. *J. Agric. Sci.* 155, 1093–1112. doi:10.1017/S0021859617000235.

Ceylan, Y., Kutman, U. B., Mengutay, M., and Cakmak, I. (2016). Magnesium applications to growth medium and foliage affect the starch distribution, increase the grain size and improve the seed germination in wheat. *Plant Soil* 406, 145–156. doi:10.1007/s11104-016-2871-8.

Costa, C. H. M., Carmeis Filho, A. C. A., Crusciol, C. A. C., Soratto, R. P., and Guimarães, T. M. (2018). Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. *Crop Pasture Sci.* 69, 488–505. doi:10.1071/CP17233.

Costa, C. H. M., and Crusciol, C. A. C. (2016). Long-term effects of lime and phosphogypsum application on tropical no-till soybean-oat-sorghum rotation and soil chemical properties. *Eur. J. Agron.* 74, 119–132. doi:10.1016/j.eja.2015.12.001.

Croft, H., Chen, J. M., Luo, X., Bartlett, P., Chen, B., and Staebler, R. M. (2017). Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Glob. Chang. Biol.* 23, 3513–3524. doi:10.1111/gcb.13599.

Crusciol, C. A. C., Marques, R. R., Carmeis Filho, A. C. A., Soratto, R. P., Costa, C. H. M., Ferrari Neto, J., et al. (2019). Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. *Nutr. Cycl. Agroecosystems* 115, 347–372. doi:10.1007/s10705-019-10017-0.

Cunningham, C. (2020). Characterization of dry spells in Southeastern Brazil during the monsoon season. *Int. J. Climatol.* 40, 4609–4621. doi:10.1002/joc.6478.

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., et al. (2010). Cadmium stress: An oxidative challenge. *BioMetals* 23, 927–940. doi:10.1007/s10534-010-9329-x.

Dejardin, A., Rochat, C., Wuilleme, S., and Boutin, J.-P. (1997). Contribution of sucrose synthase, ADP-glucose pyrophosphorylase and starch synthase to starch synthesis in developing pea seeds. *Plant, Cell Environ.* 20, 1421–1430. doi:10.1046/j.1365-3040.1997.d01-32.x.

Eekhout, T., Larsen, P., and De Veylder, L. (2017). Modification of DNA Checkpoints to Confer Aluminum Tolerance. *Trends Plant Sci.* 22, 102–105. doi:10.1016/j.tplants.2016.12.003.

Fancelli, A. L. (2015). “Ecofisiologia, fenologia e implicações básicas de manejo,” in *BORÉM, A.; GALVÃO, JCC; PIMENTEL, MA Milho: do plantio à colheita. Viçosa: Ed. UFV*, 50–76.

FAO, I. (2015). Status of the world’s soil resources (SWSR)–main report. *Food Agric. Organ. United Nations Intergov. Tech. panel soils, Rome, Italy* 650.

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C., and Rabhi, M. (2016). Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiol. Plant.* 38. doi:10.1007/s11738-016-2165-z.

Farmer, E. E., and Mueller, M. J. (2013). ROS-Mediated Lipid Peroxidation and RES-Activated Signaling. *Annu. Rev. Plant Biol.* 64, 429–450. doi:10.1146/annurev-arplant-050312-120132.

Giannopolitis, C. N., and Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 59, 309–314. doi:https://doi.org/10.1104/pp.59.2.309.

Gibbons, J. M., Williamson, J. C., Williams, A. P., Withers, P. J. A., Hockley, N., Harris, I. M., et al. (2014). Sustainable nutrient management at field, farm and regional level: Soil testing, nutrient budgets and the trade-off between lime application and greenhouse gas emissions. *Agric. Ecosyst. Environ.* 188, 48–56. doi:10.1016/j.agee.2014.02.016.

Gibbs, H. K., and Salmon, J. M. (2015). Mapping the world's degraded lands. *Appl. Geogr.* 57, 12–21. doi:10.1016/j.apgeog.2014.11.024.

Gomes-Junior, R. A., Moldes, C. A., Delite, F. S., Pompeu, G. B., Gratao, P. L., Mazzafera, P., et al. (2006). Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. *Chemosphere* 65, 1330–1337. doi:https://doi.org/ 10.1016/j.chemosphere.2006.04.056.

Gómez, R., Vicino, P., Carrillo, N., and Lodeyro, A. F. (2019). Manipulation of oxidative stress responses as a strategy to generate stress-tolerant crops. From damage to signaling to tolerance. *Crit. Rev. Biotechnol.* 39, 693–708. doi:10.1080/07388551.2019.1597829.

Gong, Z., Xiong, L., Shi, H., Yang, S., Herrera-Estrella, L. R., Xu, G., et al. (2020). Plant abiotic stress response and nutrient use efficiency. *Sci. China Life Sci.* 63, 635–674. doi:10.1007/s11427-020-1683-x.

Gratão, P. L., Monteiro, C. C., Antunes, A. M., Peres, L. E. P., and Azevedo, R. A. (2008). Acquired tolerance of tomato (Lycopersicon esculentum cv. Micro-Tom) plants to cadmium-induced stress. *Ann. Appl. Biol.* 153, 321–333. doi:https://doi.org/ 10.1016/j.chemosphere.2006.04.056.

Gupta, A., Rico-Medina, A., and Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science (80-.).* 368, 266–269. doi:10.1126/science.aaz7614.

Heanes, D. L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Commun Soil Sci Plant Anal, 10, 191-1213.

Heath, R. L., and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198. doi:https://doi.org/10.1016/0003-9861(68)90654-1.

Heinemann, A. B., Ramirez-Villegas, J., Stone, L. F., Silva, A. P. G. A., da Matta, D. H., and Diaz, M. E. P. (2021). The impact of El Niño Southern Oscillation on cropping season rainfall variability across Central Brazil. *Int. J. Climatol.* 41, E283–E304. doi:10.1002/joc.6684.

Holland, J. E., Bennett, A. E., Newton, A. C., White, P. J., McKenzie, B. M., George, T. S., et al. (2018). Liming impacts on soils, crops and biodiversity in the UK: A review. *Sci. Total Environ.* 610–611, 316–332.

doi:10.1016/j.scitotenv.2017.08.020.

Kiehl, E. J. (1979). Edaphology manual: soil-plant relationship. *Agronômica Ceres. São Paulo, Brazil Piracicaba*.

Kitao, M., Lei, T. T., and Koike, T. (1997). Effects of manganese toxicity on photosynthesis of white birch (Betula platyphylla var. japonica) seedlings. *Physiol. Plant.* 101, 249–256. doi:10.1034/j.1399-3054.1997.1010201.x.

Kleiner, K., Abrams, M., and Schultz, J. (1992). The Impact of Water & Nutrient Deficiencies on the Growth. *Tree Physiol.* 11, 271–287. doi:https://doi.org/10.1093/treephys/11.3.271.

Lammel, D. R., Barth, G., Ovaskainen, O., Cruz, L. M., Zanatta, J. A., Ryo, M., et al. (2018). Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. *Microbiome* 6, 7–9. doi:10.1186/s40168-018-0482-8.

Lichtenthaler, H. K. (1987). “Chlorophylls and carotenoids: pigments of photosynthetic biomembranes,” in *Methods in enzymology* (Elsevier), 350–382. doi:https://doi.org/10.1016/0076- 6879(87)48036-1.

Liu, H., Chen, X., Chen, R., Song, S., and Sun, G. (2008). Effects of magnesium deficiency on growth and photosynthesis of flowering Chinese cabbage. *Acta Hortic.* 767, 169–176.

Liu, Y. F., Zhang, G. X., Qi, M. F., and Li, T. L. (2015). Effects of Calcium on Photosynthesis, Antioxidant System, and Chloroplast Ultrastructure in Tomato Leaves Under Low Night Temperature Stress. *J. Plant Growth Regul.* 34, 263–273. doi:10.1007/s00344-014-9462-9.

Loix, C., Huybrechts, M., Vangronsveld, J., Gielen, M., Keunen, E., and Cuypers, A. (2018). Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Front. Plant Sci.* 9, 1–2. doi:10.3389/fpls.2018.00391.

Maia, C. E. (2012). Sampling time of banana leaves for nutritional diagnosis. *Rev. Bras. Ciência do Solo* 36, 859–864.

Malavolta, E. (1997). Avaliação do estado nutricional das plantas: princípios e aplicações/Eurípedes Malavolta, Godofredo Cesar Vitti, Sebastião Alberto de Oliveira. *Piracicaba: Potafos*.

Nelson, L. S. (1998). The Anderson-Darling test for normality. *J. Qual. Technol.* 30, 298.

Noctor, G. (2002). Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. *J. Exp. Bot.* 53, 1283–1304. doi:10.1093/jexbot/53.372.1283.

Nora, D. D., Amado, T. J. C., Nicoloso, R. da S., Mazuco, A. C. B., and Piccin, M. (2017). Mitigation of the gradient of chemical properties in the rooting zone of dystrophic oxisols by gypsum and lime inputs under a no-till system. *Rev. Bras. Cienc. do Solo* 41, 1–22. doi:10.1590/18069657rbcs20150541.

Reid, C. D., Tissue, D. T., Fiscus, E. L., and Strain, B. R. (1997). Comparison of

spectrophotometric and radioisotopic methods for the assay of Rubisco in ozone-treated plants. *Physiol. Plant.* 101, 398–404. doi:https://doi.org/10.1111/j.1399-3054.1997.tb01014.x.

Reis, A. R. dos, Lisboa, L. A. M., Reis, H. P. G., Barcelos, J. P. de Q., Santos, E. F., Santini, J. M. K., et al. (2018). Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. *Plant Physiol. Biochem.* 130, 377–390. doi:10.1016/j.plaphy.2018.07.028.

Rellán-Álvarez, R., Lobet, G., and Dinneny, J. R. (2016). Environmental Control of Root System Biology. *Annu. Rev. Plant Biol.* 67, 619–642. doi:10.1146/annurev-arplant-043015-111848.

Ritchey, K. D., Feldhake, C. M., Clark, R. B., and Sousa, D. M. G. (1995). “Improved water and nutrient uptake from subsurface layers of gypsum-amended soils,” in *Agricultural Utilization of Urban and Industrial By-Products*, ed. D. L. et al. Karlen (Madison: ASA, CSSA, and SSSA), 157–181.

Ritchey, K. D., Silva, J. E., and Costa, U. F. (1982). Calcium deficiency in clayey B horizons of savanna oxisols. *Soil Sci.* 133, 378–382.

Ritchie, S. W., Hanway, J. J., and Benson, G. O. (1993). How a corn plant develops. Special Report n. 48. Iowa State University of Science and Technology. *Coop. Ext. Serv. Ames, IA, USA*.

Rolim, G. de S., SENTELHAS, P. C., and BARBIERI, V. (1998). Planilhas no ambiente EXCEL TM para os cálculos de balanços hídricos: normal, sequencial, de cultura e de produtividade real e potencial. *Rev. Bras. Agrometeorol.* 6, 133–137.

Roth, C. H., and Pavan, M. A. (1991). Effects of lime and gypsum on clay dispersion and infiltration in samples of a Brazilian Oxisol. *Geoderma* 48, 351–361. doi:10.1016/0016-7061(91)90053-V.

Shoemaker, H. E., McLean, E. O., and Pratt, P. F. (1961). Buffer Methods for Determining Lime Requirement of Soils With Appreciable Amounts of Extractable Aluminum. *Soil Sci. Soc. Am. J.* 25, 274–277. doi:10.2136/sssaj1961.03615995002500040014x.

Silva, V. M., Rimoldi Tavanti, R. F., Gratão, P. L., Alcock, T. D., and Reis, A. R. dos (2020). Selenate and selenite affect photosynthetic pigments and ROS scavenging through distinct mechanisms in cowpea (Vigna unguiculata (L.) walp) plants. *Ecotoxicol. Environ. Saf.* 201. doi:10.1016/j.ecoenv.2020.110777.

Song, Y., Miao, Y., and Song, C. P. (2014). Behind the scenes: The roles of reactive oxygen species in guard cells. *New Phytol.* 201, 1121–1140. doi:10.1111/nph.12565.

Stein, O., and Granot, D. (2019). An overview of sucrose synthases in plants. *Front. Plant Sci.* 10, 1–14. doi:10.3389/fpls.2019.00095.

Thornthwaite, C. W., and Mather, J. (1955). JR The water balance. *Centerton, NJ Drexel*.

Unicamp (2020). Center of Meteorological and Climatic Research Applied to Agriculture. Botucatu: Municipalities climate of São Paulo State. Available at: www.cpa.unicamp.br/outras- informacoes/clima_muni_086.html.

USDA (2014). “Keys to soil taxonomy,” in (Washington, DC: US Gov. Print. Office), 327–328.

van Raij, B., Cantarella, H., Quaggio, J. A., and Furlani, A. M. C. (1997). *Recomendações de adubação e calagem para o Estado de São Paulo*. Instituto Agronômico/Fundação IAC Campinas.

van Raij, B., Quaggio, J. A., Cantarella, H., and Abreu, C. A. (2001). Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. *Análise química para avaliação da Fertil. solos Trop.*, 5–39.

Wang, Q., Yang, S., Wan, S., and Li, X. (2019). The significance of calcium in photosynthesis. *Int. J. Mol. Sci.* 20, 1–14. doi:10.3390/ijms20061353.

Wellburn, A. R. (1994). The spectral determination of chlorophyll a and b, as well as carotenoids using various solvents with spectrophotometers of different resolution. *J. Plant Physiol* 144, 307–313.

Zoca, S. M., and Penn, C. (2017). An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. *Adv. Agron.* 144, 1–44. doi:10.1016/bs.agron.2017.03.001.

SUPPLEMENTARY MATERIAL

Table S1. Crops growing and treatments application scheme during the experimental period (from 2002 to 2018).

Growing season	Seasons		Treatments[†]
	Summer crops	Autumn-Winter-Spring crops	
2002/2003	*Oriza sativa*	*Avena strigosa*	Lime: 2.7 Mg ha^{-1} (71% ECCE[‡]) Phosphogypsum: 2.1 Mg ha^{-1}
2003/2004	*Phaseolus vulgaris*	*Avena strigosa*	-
2004/2005	*Arachis hypogaea*	*Avena sativa*	Lime: 2.0 Mg ha^{-1} (71% ECCE) Phosphogypsum: 2.1 Mg ha^{-1}
2005/2006	*Arachis hypogaea*	*Avena sativa*	-
2006/2007	*Zea mays* intercropped with *Urochloa brizantha* (cv. Marandu)	*Urochloa brizantha* (cv. Marandu) (after *Zea mays* harvest)	-
2007/2008	*Zea mays* intercropped with *Urochloa brizantha* (cv. Marandu)	*Urochloa brizantha* (cv. Marandu) (after *Zea mays* harvest)	-
2008/2009	*Glycine max*	*Avena strigosa*	-
2009/2010	*Glycine max*	*Sorghum vulgare*	-
2010/2011	*Zea mays*	*Crambe abyssinica* / *Vigna unguiculata*	Lime: 2.0 Mg ha^{-1} (88% ECCE) Phosphogypsum: 2.1 Mg ha^{-1}
2011/2012	*Zea mays*	*Crambe abyssinica* / *Vigna unguiculata*	-
2012/2013	*Pennisetum glaucum*	*Triticum aestivum*	-
2013/2014	*Phaseolus vulgaris*	*Triticum aestivum*	-
2014/2015	*Phaseolus vulgaris*	*Urochloa brizantha* (cv. Marandu) (1st growing season)	-
2015/2016	*Urochloa brizantha* (cv. Marandu) (2nd growing season)	*Urochloa brizantha* (cv. Marandu) (3rd growing season)	-
2016/2017[§]	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	Lime: 13 Mg ha^{-1} (69% ECCE) Phosphogypsum: 10 Mg ha^{-1}
2017/2018	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	-

[†]The reapplications in October 2004, 2010 and 2016 were performed when base saturation reached ≤ 50%.
[‡]ECCE: effective calcium carbonate equivalent
[§]Micronutrients applied in total area

Table S2. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG)] for soil chemical properties in stratified layers (0.0–1.0 m depth).

Soil properties	Soil depth (m)					
	0.0–0.1	0.1–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
pH	<0.001	<0.001	0.0271	<0.001	0.0016	0.0048
LSD†	0.27	0.33	0.48	0.14	0.18	0.16
CV‡ (%)	3.32	4.51	6.98	2.10	2.76	2.59
Ca^{2+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	2.96	2.25	2.13	1.08	1.15	0.50
CV (%)	4.20	5.22	5.87	5.01	9.12	5.80
Mg^{2+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	1.88	1.34	1.87	1.86	1.72	0.49
CV (%)	6.59	7.64	14.4	15.3	16.5	15.4
BS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	3.62	5.07	4.73	2.07	1.53	0.55
CV (%)	4.32	7.71	9.98	6.20	6.94	4.16
Al^{3+}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	1.16	5.94	1.61	3.18	3.83	3.97
CV (%)	15.3	29.4	11.7	16.2	16.0	16.2
SO_4^{2-}-S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	3.68	5.42	7.74	7.77	7.58	10.6
CV (%)	10.6	12.0	12.5	11.0	9.87	13.7

Exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminum (Al^{3+}), base saturation (BS) and sulfate (SO_4^{2-}-S). †Low significant difference (LSD), ‡coefficient of variation (CV)

Table S3. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG)] for soil chemical properties at 0.0–0.2 m depth.

Treatments	SOM	P	Fe	Mn	Cu	Zn
p value	<0.001	<0.001	<0.001	<0.001	0.2023	0.0161
LSD†	1.59	4.14	3.16	5.05	0.50	0.76
CV‡ (%)	3.61	7.44	8.61	9.64	15.8	14.7

Soil organic matter (SOM), phosphorus (P), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn)
†Low significant difference (LSD), ‡coefficient of variation (CV)

Table S4. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for maize root dry matter and root dry matter distribution in stratified layers (0.0–1.0 m depth).

Treatments	Soil depth (m)					
	0.0–0.1	0.1–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
	Root dry matter					
SA* (2017)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD†	0.0652	0.0570	0.0482	0.0364	0.0338	0.0267
SA (2018)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.0539	0.0506	0.0423	0.0320	0.0237	0.0205
CV‡ (%; 2017)	8.54	7.55	9.75	10.90	9.04	8.39
CV‡ (%; 2018)	6.03	5.73	7.13	7.83	6.53	7.23
	Root dry matter distribution					
SA (2017)	<0.001	<0.001	0.3454	<0.001	<0.001	<0.001
LSD	2.28	1.73	1.35	1.49	1.37	0.96
SA (2018)	<0.001	<0.001	0.4298	<0.001	<0.001	<0.001
LSD	3.84	2.96	1.43	1.13	1.09	0.75
CV‡ (%; 2017)	4.27	3.17	7.68	5.58	9.37	9.67
CV‡ (%; 2018)	6.49	3.89	8.78	7.36	11.37	8.43

*Soil amendments (SA), †Low significant difference (LSD), ‡coefficient of variation (CV)

Table S5. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for nutritional status of maize.

Treatments	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn
SA* (2017)	<0.001	0.1267	0.6117	0.0037	<0.001	<0.001	0.0236	0.0094	0.6967	<0.001
LSD†	3.87	0.165	2.78	1.09	1.26	0.49	74.1	5.08	4.58	17.7
SA (2018)	<0.001	0.0235	0.041	0.1266	<0.001	<0.001	0.0016	0.0120	0.1156	0.0018
LSD	3.90	0.23	2.60	0.59	1.14	0.59	41.5	5.08	2.91	16.2
CV‡ (%; 2017)	7.15	4.43	8.35	21.4	20.4	11.6	21.0	13.1	22.6	17.8
CV (%; 2018)	8.55	7.02	9.00	14.8	20.5	18.2	13.0	13.9	15.4	19.3

*Soil amendments (SA), Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). †Low significant difference (LSD), ‡coefficient of variation (CV).

Table S6. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level of experimental factors [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) × two growing seasons] for maize physiological, biochemical, and agronomic parameters.

Treatments	Chl *a*	Chl *b*	Total chl	Carot	*A*	*gs*	*E*	*ic*	WUE	Rubisco
SA* (2017)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0011	<0.001	<0.001	-
LSD	4.21	0.93	5.13	0.66	2.46	0.48	1.64	8.38	1.23	-
SA (2018)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	3.15	0.77	3.93	0.51	1.80	0.49	0.40	9.51	0.95	0.32
CV‡ (%; 2017)	20.1	20.6	20.2	10.3	5.25	11.0	19.4	7.26	11.8	-
CV (%; 2018)	20.7	20.8	20.7	10.5	5.35	12.6	5.49	7.34	9.73	4.76

	Sucrose	Susy	H_2O_2	MDA	SOD	CAT	GR	APX	SDM	GY
SA (2017)	<0.001	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.0176	<0.001	<0.001
LSD	1.46	-	0.98	1.21	5.79	0.63	0.04	0.60	1.14	0.79
SA (2018)	0.0156	<0.001	<0.001	<0.001	<0.001	<0.001	0.0023	0.0011	<0.001	<0.001
LSD	2.34	2.64	1.30	1.59	7.94	1.23	0.34	0.29	0.30	0.21
CV‡ (%; 2017)	2.38	-	5.99	6.09	8.91	11.6	15.1	9.06	9.90	7.55
CV (%; 2018)	3.88	8.66	5.91	5.92	9.12	15.5	28.5	4.71	5.85	5.11

*Soil amendments (SA), Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), total chlorophyll (Total chl), carotenoids (Carot), Net photosynthesis rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*ic*), transpiration (*E*), water use efficiency (WUE), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), shoot dry matter (SDM) and grain yield (GY). †Low significant difference (LSD), ‡coefficient of variation (CV).

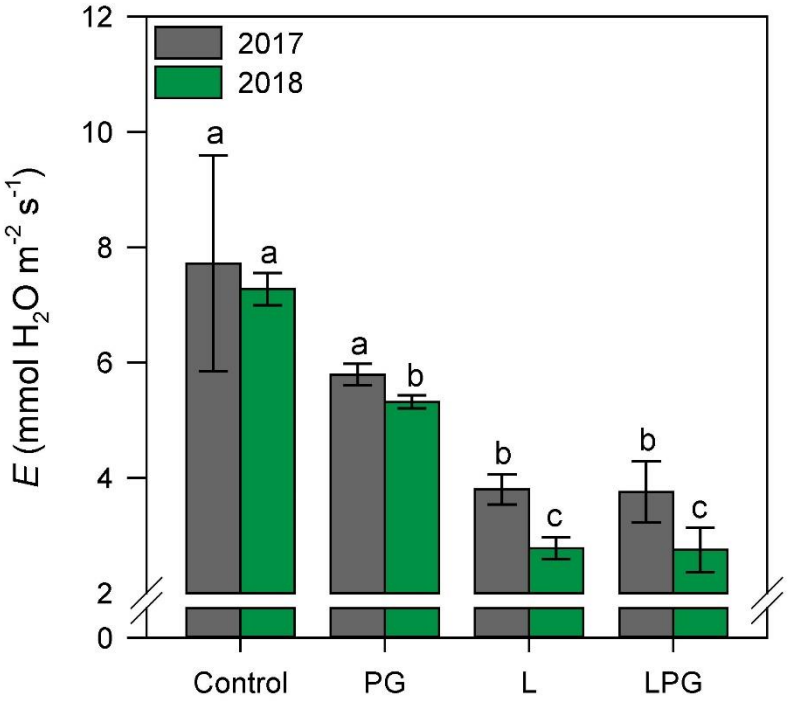


Figure S1. Leaf transpiration rate (*E*) in maize leaves as affected by surface-applied lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG) treatments. Different lower-case letters indicate significant differences between treatments for each growing season by Student's t-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

CHAPTER 3

MODULATION OF THE SOIL MICROBIOME BY LONG-TERM CA-BASED SOIL AMENDMENTS BOOSTS SOIL ORGANIC CARBON AND PHYSICOCHEMICAL QUALITY IN A TROPICAL NO-TILL CROP ROTATION SYSTEM[3]

Published in: Bossolani JW, Crusciol CAC, Leite MFA, et al (2021) Modulation of the soil microbiome by long-term Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. Soil Biol Biochem 156:. https://doi.org/10.1016/j.soilbio.2021.108188

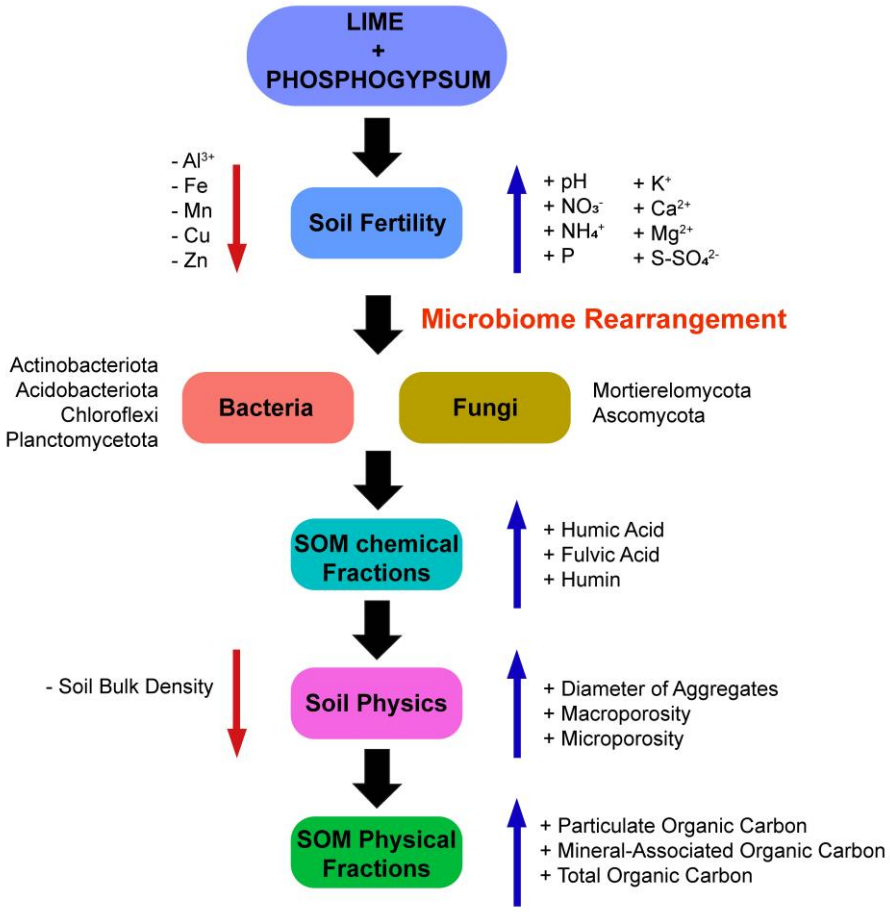
Abstract

Unsustainable agricultural management practices such as non-conservationist tillage and overuse of fertilizers result in soil acidity and, in turn, soil degradation due to reduced carbon (C) concentrations and nutrient availability and increased aluminum toxicity. Application of lime (L) and phosphogypsum (PG) can overcome these constraints and improve soil quality, but the long-term effects of these amendments on both abiotic and biotic soil properties are not known, particularly when applied in combination. Here, we evaluated the effects of L (acidity corrective), PG (soil conditioner), and their combination (LPG) on soil organic matter (SOM) transformations, soil chemical and physical properties, and microbiome assembly in a long-term experiment under a no-till crop rotation system in a tropical soil. The Ca-based soil amendments increased C concentrations (labile and stable fractions), improved soil physicochemical properties, and changed the associations between several bacterial and fungal groups. Contrary to expectations, the acidic soil amended with PG exhibited greater number of significant shifts in the bacterial community than soil amended with L or LPG, as well as higher soil bulk density. By contrast, the fungal community underwent greater shifts in soil amended with L or LPG, which had higher macroporosity. L and LPG amendment shaped the fungal community and rearranged the SOM fractions at similar rates, suggesting an essential role of the altered fungi in SOM transformation. In addition, combining L with PG increased the relevance of many low-abundance microorganisms, especially fungi, compared with the control, indicating an increase in their ecological role in the soil. Finally, by applying general joint attribute modeling and sensitivity analysis, we determined that soil fertility increased most in LPG-amended soil, as the ensuing changes in the bacterial and fungal communities resulted in improved SOM fractions, soil physical characteristics and, ultimately, soil quality.

Keywords: pH; long-term field experiment; soil organic matter transformation; bacterial community; fungal community; tropical soil; no-till system.

[3] Chapter written in accordance with the rules of Soil Biology and Biochemistry

Graphical abstract



3.1 INTRODUCTION

The processes that govern soil acidification are global drivers of soil degradation (low productive capacity) (von Uexküll and Mutert, 1995; Gibbs and Salmon, 2015; Meng et al., 2019). Soil acidification is a natural phenomenon resulting from the weathering of parent material with low exchangeable bases [e.g., calcium (Ca^{2+}) and magnesium (Mg^{2+})] but is worsened by unsustainable soil management practices such as non-conservationist tillage, overuse of ammonium-based fertilizers, removal of cations by crop harvesting, and release of organic acids from decomposing crop residues (Bian et al., 2013; Tiritan et al., 2016; Pulido-Moncada et al., 2018). The same agricultural mismanagement practices that worsen soil acidity and fertility also negatively impact soil organic matter (SOM) (Fageria and Baligar, 2008; Carmeis Filho et al., 2017b). Low SOM levels in soils promote leaching of cations, further contributing

to soil acidification and severely restricting crop production (Evans et al., 2012; Castellano et al., 2015; Paradelo et al., 2015; Carmeis Filho et al., 2017b).

In addition to deteriorating abiotic soil properties (chemical and physical), soil acidity disrupts soil microbial community structure, abundance, activities and functions (Kuramae et al., 2010, 2012; Navarrete et al., 2013). The soil microbiome is one of the main reservoirs of diversity in the biosphere and plays an essential role in agroecosystem functioning, including SOM decomposition and nutrient cycling (Kušliene et al., 2014). Low soil pH reduces carbon (C) concentrations and nutrient availability and increases the toxicity of certain elements [e.g., aluminum (Al^{3+}) and manganese (Mn)], thus directly affecting microbial growth (Bowman et al., 2008; Tian et al., 2019). Additionally, the high concentration of hydrogen (H^{+}) in acidic soils likely reduces microbial biomass and physiology (Meng et al., 2019; Tian et al., 2019).

Alleviating these deleterious effects requires sustainable agricultural practices that increase soil productive capacity while providing important ecosystem services, such as increasing the stock of nutrients and C in the soil and improving activities in belowground processes (De Tender et al., 2019). Liming is a traditional practice to mitigate the negative effects of acidity on soil and crops, particularly in tropical soils (Carmeis Filho et al., 2017a; Crusciol et al., 2019; Bossolani et al., 2020a, 2020b). Liming increases nutrient availability and provides extra nutrient inputs (i.e., Ca^{2+} and Mg^{2+}) (Bossolani et al., 2020b), thereby improving soil fertility and resources for plant and microbial growth (Liu et al., 2018; Guo et al., 2019a ; Bossolani et al., 2020a; Jha et al., 2020).Thus, beyond neutralizing acidic soils, liming benefits soil health (Holland et al., 2018) to promote the grain and biomass yield of arable and grassland crops. Due to its low solubility, lime (L) is typically applied to the soil and subsequently incorporated to increase the reaction efficiency (Bossolani et al., 2018; Crusciol et al., 2019; Fontoura et al., 2019). However, the emergence of no-till systems (NTS) has led to the introduction of superficial liming (lime applied to the soil surface, without subsequently incorporation through plowing and harrowing), the effects of which on soil acidity are unclear. To circumvent the limitations of superficial liming, a few recent studies have evaluated the application of phosphogypsum (PG, composed primarily of $CaSO_4.2H_2O$), a by-product of phosphoric acid production, in combination with L (Carmeis Filho et al., 2017b; Crusciol et al., 2019; Bossolani et al., 2020a). Unlike L, PG does not correct soil acidity but is more soluble in soil and can potentially reduce

Al^{3+} concentrations and increase Ca^{2+} availability. Thus, PG is useful as a supplement to improve soil quality under NTS (Zoca and Penn, 2017; Bossolani et al., 2018).

The main challenge in using Ca-based soil amendments is achieving a balance between crop productivity and soil quality that provides a sustainable production system (Yun et al., 2016; Holland et al., 2018; Liu et al., 2018). The impacts of Ca-based soil amendments on agriculture are wide-ranging, and previous work has focused on isolated effects on soil properties and short-term impacts. Short-term research is insufficient to assess ecosystem properties, such as soil structure, C stocks, and microbiota, that may take decades to stabilize, and results obtained before 15 years have elapsed are susceptible to misinterpretation (Cusser et al., 2020). The few studies that have examined long-term agricultural management with L and PG have shown increases in soil physicochemical quality (Briedis et al., 2012; Carmeis Filho et al., 2018). However, we still lack an understanding of how combined Ca-based soil amendments (L + PG) affect soil chemical and physical factors, C sequestration, and microbial communities and how these soil factors are associated with and affect the soil microbiome.

To address this gap, we investigated the legacy of long-term L and PG amendments alone or in combination in a no-tillage intercropping system by answering the following two questions:

1) How did 17 years of amendment with lime, phosphogypsum or both change the abiotic properties (chemical and physical) and biotic communities (bacteria and fungi) of the soil?

2) How were the changes in microorganisms associated with changes in soil chemical and physical properties?

We hypothesized that the type of soil amendment would affect soil fertility, soil physical structure and soil organic matter fractions and these factors would drive different associations of bacterial and fungal groups.

3.2 MATERIAL AND METHODS

3.2.1 Field description

The experiment was conducted in a 17 years-old long-term experimental field (Soratto and Crusciol, 2008) in Brazil (22° 83' 3" S, 48° 42' 64" W, elevation 765 m

above sea level). The soil is a sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2014). The soil physicochemical characteristics prior the experiment (2002) were soil pH (0.01 M $CaCl_2$ suspension): 4.2, soil organic matter (SOM): 21 g kg^{-1}; K^+: 1.2 $mmol_c$ kg^{-1}; Ca^{2+}: 14 $mmol_c$ kg^{-1}; Mg^{2+}: 5 $mmol_c$ kg^{-1}; total acidity at pH 7 (H+Al): 38.8 $mmol_c$ kg^{-1}, cation exchange capacity (CEC): 58 $mmol_c$ kg^{-1}; BS: 35%; aluminum saturation (AS): 65% (see the complete physicochemical characterization in Table S1). According to the Köppen-Geiger's climatic classification, the region corresponds a mesothermic type (Cwa) with a humid subtropical dry winter and hot summer (Alvares et al., 2013). The long-term average (1956-2019) annual temperature is 26.1 °C maximum and 15.3 °C minimum with a mean annual precipitation of 1359 mm (Unicamp, 2020).

3.2.2 Experimental setup, treatments and cropping history

The experimental design was a randomized complete block with four treatments and four replications. The treatments were (*i*) natural soil conditions (control treatment with no lime and no phosphogypsum amendment), (*ii*) lime (L) (13 Mg ha^{-1}); (*iii*) phosphogypsum (PG) (10 Mg ha^{-1}); (*iv*) combined L plus PG (LPG). Fertilizer inputs (N-P_2O_5-K_2O) occurred in all plots, including the control treatment, in all crops established in this experiment over the 17 years. A schematic graph representing the crop system, soil amendment application and sampling is illustrated in Fig. 1. During the study period (2002–2019), the treatments were applied four times (2002, 2004, 2010 and 2016), and different crops were cultivated in season and off season from 2002 to 2019. The treatments were applied whenever the base saturation (BS) of the standard treatment (exclusive L) reached value ≤ 50% in the soil (0.00–0.40 m). Soil fertility was monitored through annual sampling. This study had maize (*Zea mays* L.) intercropped with ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (Syn. *Brachiaria ruziziensis* Germ. & Evrard)] sown in agricultural year of 2019. Details of the previous crop sequences and in each reapplication, as well as the characteristics of the applied amendments are shown in Table S2. Since the experiment was initiated, the treatments were performed superficially, except for the first application in October 2002, when no-tillage system was initiated.

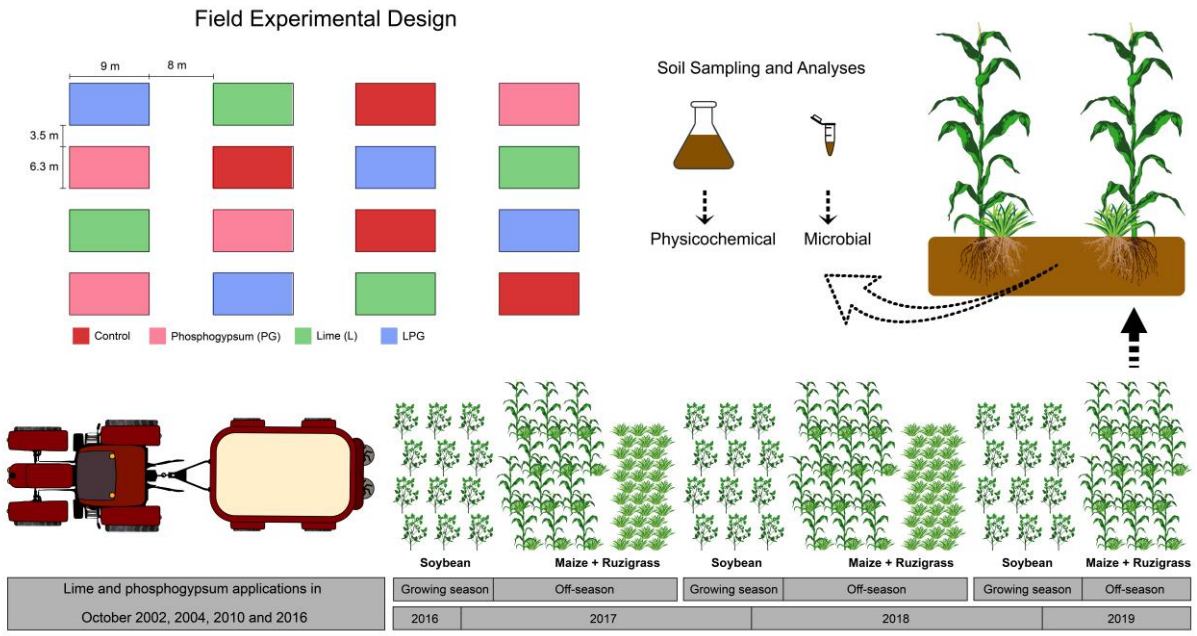


Figure 1. Schematic graph representing the field experimental design, crop system, soil amendment application and sampling.

The water content of L and PG amendments were determined for the calculations of the correct rate to be applied on soil, which calculations considering both amendments with 0 g kg^{-1} of water content. The composition of amendments applied in last reapplication were measured: effective calcium carbonate equivalent (ECCE): 69%, CaO: 310 g kg^{-1} and MgO: 140 g kg^{-1} for L and CaO: 280 g kg^{-1}, S: 150 g kg^{-1} for PG. All the calculations of recommended rate of L and PG are shown in the Supplementary Material and Methods.

The bulk soil samples were collected between maize + ruzigrass rows at 0.00–0.10 m layer from a no-till maize intercropped with ruzigrass system. The samples were collected at V_{10} phenological maize stage (Ritchie et al., 1993) in June 2019. For physicochemical and organic matter fractionation, samples were air-dried and sieved (2 mm sieve) and for molecular analysis, aliquots of 80 g fresh soil were stored at -20 °C for subsequent DNA extraction.

3.2.3 Soil sampling and analysis

3.2.3.1 Soil physicochemical properties

Soil samples were processed according to the standard methods for Brazilian tropical soils (van Raij et al., 2001). Seventeen years after treatment establishment (there years after last treatments reapplication), ten individual soil subsamples of each plot were randomly taken at 0.00–0.10 m depth with a galvanized-steel probe (69-mm-diameter) to compose the sample. The samples were separated into two subsamples: subsample 1 to air dry and then homogenized for further chemical analysis (except NO_3^- and NH_4^+) and subsample 2 to store at -20 ^oC for 15 days until NO_3^- and NH_4^+ determination could be performed. The soil pH values were measured in 0.01 M $CaCl_2$ (the most stable pH used in soil analysis) (van Raij et al., 2001).The exchangeable cations (K^+, Ca^{2+}, and Mg^{2+}) and available P-phosphate were extracted using ion exchange resins and, in the extract, P was determined colorimetrically, and cations by atomic absorption spectrometry (Shimadzu AA-7000) (van Raij et al., 2001). Soil sulfur-sulfate ($S\text{-}SO_4^{2-}$) extraction were performed by calcium phosphate extraction at 0.01 mol L^{-1} in a 1:2.5 soil/solution ratio and later determined by the turbidimetric method using $BaSO_4$ (Bardsley and Lancaster, 1960). The cationic micronutrients (Fe, Mn, Cu and Zn) were extracted in a mixture DTPA 0.005 M + TEA 0.1 M + $CaCl_2$ 0.01 M, and determined by atomic absorption spectrometry (van Raij et al., 2001). The total acidity at pH 7.0 (H+Al) was estimated by the Shoemaker-McLean-Pratt buffer solution method (Shoemaker et al., 1961). The exchangeable Al^{3+} was extracted with neutral 1 mol L^{-1} KCl at 1:10 soil/solution ratio and determined by titration with a 0.025 mol L^{-1} NaOH solution. After extraction of KCl solution, the content of ammonium (NH_4^+) and nitrate (NO_3^-) was also determined by colorimetry by sodium salicylate method and vanadium chloride reduction respectively (Mulvaney et al., 1996). Total inorganic nitrogen (Ni) was obtained by summing the measured concentrations of mineral forms (NH_4^+ and NO_3^-).For the soil water-stable aggregate (WSA) analysis, undisturbed soil monolith samples (~ 200 g each sample) were collected and air dried, slightly sieved in an 8.0-mm sieve, and retained on the 4.0-mm sieve. A 25-g aliquot of the retained aggregate fraction (air-dried) was gently wetted by a spray-bottle with water taken to wet-sieving for 15 min using a nest of sieve with 4.00, 2.00, 1.00, 0.50, 0.25, and 0.105-mm mesh sizes coupled to mechanical equipment adjusted to 31 vertical oscillations min^{-1} (Yoder, 1936). The fractions obtained (retained on each sieve) were dried in a forced-air oven at 42 °C for 72 h and weighed afterwards. Through the initial moisture content of each soil fraction and its respective weight, the values were adjusted to 0 g kg^{-1} of water (dry soil weight). These results were used to calculate the mean weight

diameter (MWD) according to the methodologies proposed by Kemper and Chepil (2015). In addition, the undisturbed soil samples were placed to gradually saturate with water over 48 h. Then, all saturated samples were weighed and taken to Richard's pressure chamber on porous plates under -0.006 MPa tension (Klute, 1986) until they stabilized. Subsequently, the samples were weighed and dried in a forced-air oven at 105 °C for 60 h. The total porosity was calculated by the difference between the weights of the water-saturated and dried samples. Macroporosity was determined as the difference between the water content of the water-saturated samples and after being subjected to the Richard's pressure chamber at -0.006 MPa tension. Microporosity was calculated as the difference between the total porosity and macroporosity (Smith and Mullins, 1991).

3.2.3.2 Soil organic matter physicochemical fractionation

In the same soil samples used for chemical analysis, the total organic carbon (TOC), particulate organic carbon (POC) and mineral-associated organic carbon (MOC) were also determined. The TOC was measured by an elemental analyzer (LECO-TruSpec1 CHN, Leco Corp., St. Joseph, MI, USA). Physical fractionation of soil organic matter was performed according to Cambardella and Elliot (1994) method. A 20 g of each soil sample was homogenized in 80 mL of $(NaPO_3)_6$ solution at 5 g L^{-1}. During 16 h, the samples were horizontally shaken and then sieved through 0.053-mm mesh using deionized-water. The remaining material was dried at 40 °C in a forced-air oven and grounded with a porcelain mortar and pestle for homogenization. The POC (defined as C content in the 0.053–2 mm soil fraction) were measured via dry combustion with an elemental analyzer. Mineral-associated organic carbon was calculated using the difference between total organic carbon and particulate organic carbon.

Humic substances were obtained by chemical fractionation of organic matter (Ciavatta et al., 1990), in which Supelite DAX-8 resin was used to separate non-humified substances from humic fractions. In 10 g of each soil sample, the humic substances were extracted with 100 mL of 0.1 mol NaOH plus 0.1 mol $Na_4P_2O_7$ solution and N_2 bubbling for 2 min. The samples were shaken for 2 h at 160 oscillations min^{-1} followed by centrifugation for 25 min at 14,000 RPM. The suspension was filtered in a 0.45-mm Millipore filter and added the humin fraction. The remaining fraction (humin

and minerals) were stored for subsequent C analysis. The humic acid (HA) fraction was obtained by adding 0.5 mol H_2SO_4 to the filtered solution until it reached pH ≤ 2.0, followed by centrifugation at 9,000 RPM for 25 min; then, the precipitate was eluted in 20 ml of 0.3 mol NaOH and the C content was determined. The fulvic acid (FA) fraction was determined through the previously filtered supernatant into a plastic column containing Supelite DAX-8 resin. The retained fraction was eluted with 20 mL of 0.5 mol NaOH and taken to C determination. The organic carbon of humin (C-HU), humic acids (C-HA) and fulvic acids (C-FA) was quantified by dichromate oxidation and colorimetric determination of reduced chromium, following Heanes (1984), using sucrose (4.754 g L^{-1}) containing 2 mg organic C mL^{-1}. The organic carbon was measured by spectrophotometer at 600 nm.

3.2.3.3 Soil DNA extraction

Total soil DNA was extracted from 250 mg of each replicate soil sample using the PowerLyzer Soil DNA Isolation Kit (MOBIO laboratories, Inc.), according to manufacturer's protocol. A control DNA extraction (no soil) was carried out to check possible contamination from soil DNA extraction kit reagents. Soil DNA quantities and qualities were determined using an ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The extracted DNA was also visualized on 1% (w/w) sodium boric acid agarose gel electrophoresis. The purified DNA was stored at -80 °C until further analyses.

3.2.3.4 Amplification, sequencing and sequence processing of 16S rRNA and ITS data

Amplicon library preparation and high-throughput sequencing were performed using the DNA extracted from each soil sample. Specific regions of the gene were chosen for the sequencing of bacteria and fungus. For bacteria, we used the variable V3-V4 region amplified with forward primer 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and the reverse primer 806R (5′-G GACTACHVGGGTWTCTAAT-3′) with sizes of ~ 300–350 bp. For fungi, primers (5′-CTTGGTCATTTAGAGGAAGTAA-3′) as forward and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) as reverse were used to amplify the internal transcribed spacer region (ITS). For bacteria and fungi sequencing, primers

contained multiplex tags for sample identification. The samples were sequenced on the Illumina MiSeq System (Illumina MiSeq PE250) at McGill University and Génome Québec Innovation Center (Montréal, Québec, Canada). The sequences were deposited in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under the accession number PRJEB40513. The bacterial 16S rRNA and fungal ITS sequences were processed, demultiplexed and quality control using DADA2 pipeline (Callahan et al., 2017) in R environment (Team, 2015) with the “consensus” method, to remove any remaining chimeric and low-quality sequences. The taxonomic identification (with 99% of similarity) was performed using SILVA database (v. 138) (Quast et al., 2013) for bacterial and unite (v. 8.2) (Kõljalg et al., 2013) for fungal profiles; the taxonomic matrices (from amplicon sequence variant – ASV) generated were used for further statistical analyzes.

3.2.4 Statistical Analyses

All statistical analyses were conducted in R Studio version 1.3.959 running R version 4.02 (Team, 2015) using different packages. Generalized Joint Attribute Modeling (GJAM) package (Clark et al., 2017) was used to estimate the effects of Ca-based soil amendments. From the model we extracted the regression coefficients for each treatment to identify shifts in the microbial community (bacteria and fungi) and soil factors in different treatments. Model diagnosis evaluated the Markov Chain Monte Carlo (MCMC) to check when the estimated coefficients reached a stable value (after 10,000 simulations). The regression coefficients obtained were visualized via clusters tree using WARD distance. Same coefficients were used on PCA plot analysis to explore communities’ similarities between treatments plot to highlight shifts as induced by the different Ca-based soil amendments. Besides the intensity of the shifts in the relative abundance of the soil microbiome provided by the GJAM regression coefficients, the relevance of the different microbes for the soil community was evaluated in terms of the centered log-ratio (CLR) transformed abundance (Leite and Kuramae, 2020). CLR transformation informs the relevance of the different microbial groups (in our case both bacteria and fungi) as a proportion of the sample’s average. We used this transformation to classify the soil microbes as originally high abundant or low abundant based on the log-fold differences in relation to the average, which in the CLR-transformed data corresponds to the zero value.

3.3 RESULTS

3.3.1 Soil physicochemical parameters

The Ca-based soil amendments significantly changed ($P < 0.001$) all soil fertility parameters (Table 1; Table S3). The direct effect of L, alone or in combination with PG, increased pH by 45-50% and reduced Al^{3+} availability by ~90% compared with the control treatment. The cascade effects of changes in soil pH increased the availability of all soil macronutrients; however, the combined treatment (LPG) was more efficient than L alone in increasing the availability of NO_3^-, NH_4^+, Ca^{2+} and S-SO_4^{2-}. The most prominent effects of the Ca-based soil amendments compared with the control were observed for Ca^{2+} availability, which increased by 4.6- and 6.2-fold when L and LPG were applied, respectively, and for Mg^{2+}, which increased ~2-fold when L or LPG was applied. The content of S-SO_4^{2-} increased by 1.5-, 1.2- and 2.4-fold compared with the control when PG, L and LPG were applied, respectively. Due to the increase in soil pH, the availability of micronutrients (Fe, Mn, Cu and Zn) decreased by 30-50% compared with the control when L or LPG was applied. The Ca-based soil amendments also changed soil physical attributes (Table 1, Table S3), except microporosity (mP). Compared with the control, applying L (alone or with PG) increased the mean weight diameter (MWD) of soil aggregates by 22-28% and decreased the soil bulk density (SBD) by 14-18%. Additionally, the soil macroporosity (MP) was 44% higher compared with the control when LPG was applied, while L increased MP by ~11% and PG had no significant effect.

Table 1. Soil fertility (with available nutrients) and physical parameters of soil long-term amended with phosphogypsum (PG), lime (L) and combined lime and phosphogypsum (LPG) applied in soil surface.

Soil parameters†		units	Control	PG	L	LPG
Soil fertility	pH $_{(CaCl_2)}$	−	4.09 b	4.32 b	5.99 a	6.08 a
	Al^{3+}	$mmol_c\ kg^{-1}$	11.1 a	5.78 b	1.07 b	1.00 b
	NO_3^-	$mg\ kg^{-1}$	22.9 c	24.5 c	30.0 b	32.5 a
	NH_4^+	$mg\ kg^{-1}$	15.2 c	16.9 c	23.6 b	30.0 a
	P $_{(resin)}$	$mg\ kg^{-1}$	26.7 c	35.4 b	47.5 a	50.2 a
	K^+	$mmol_c\ kg^{-1}$	2.55 b	2.76 b	4.27 a	4.53 a
	Ca^{2+}	$mmol_c\ kg^{-1}$	11.0 d	45.0 c	61.8 b	78.5 a
	Mg^{2+}	$mmol_c\ kg^{-1}$	8.61 b	10.4 b	25.7 a	26.6 a
	$S\text{-}SO_4^{2-}$	$mg\ kg^{-1}$	9.40 a	21.2 b	23.6 b	32.4 a
	Fe	$mg\ kg^{-1}$	38.9 a	36.1 b	19.0 c	17.3 d
	Mn	$mg\ kg^{-1}$	49.5 a	38.7 b	27.9 c	26.3 c
	Cu	$mg\ kg^{-1}$	3.00 a	2.77 b	1.80 c	1.52 d
	Zn	$mg\ kg^{-1}$	4.12 a	3.85 b	2.96 c	2.50 d
Soil physics	MWD	mm	2.10 b	2.26 b	2.57 a	2.70 a
	SBD	$kg\ dm^{-3}$	1.87 a	1.71 b	1.60 c	1.54 c
	mP	%	30.7 a	29.2 a	32.6 a	32.0 a
	MP	%	9.60 bc	8.07 c	10.6 b	13.8 a

Different lower-case letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. †Mean weight diameter of soil aggregates (MWD), soil bulk density (SBD), microporosity (mP) and macroporosity (MP).

3.3.2 Labile and stable C fractions and aboveground dry matter production by maize and ruzigrass

The different lability fractions of soil organic carbon (SOC) changed significantly ($P < 0.001$) (Fig. 2; Table S3) after application of the Ca-based soil amendments four times over 17 years. Compared with control, LPG-amended soil presented the greatest increases in SOM physical fractions. Carbon present in particulate organic carbon (POC) increased by 35%, whereas in mineral-associated organic carbon (MOC) increased by 43%, and in total organic carbon (TOC) increased by 42% (Fig. 2A-C). Regarding to the SOM chemical fractions, humic acid (HA), fulvic acid (FA) and humin (HU) also changed significantly ($P < 0.001$) in response to Ca-based soil amendments (Fig. 2; Table S3). Similar increases in the C concentration from the HA and FA fractions were observed in L and LPG, whereas PG and the control treatments exhibited similar lower values of C concentration from these fractions (Fig. 2D and E). The C content from HU, was higher when PG was added to L (LPG), suggesting a synergetic effect of these two Ca-based soil amendments on SOM quality (Fig. 2F). Compared with the control, the C content from the HA increased by 27% in LPG and

by 26% in L treatments. Carbon in FA fraction increased by 38% in LPG and 30% in L, whereas the C content in HU fraction, increased by 72% in LPG and 40% in L treatment. Interestingly, the ratios between SOM chemical fractions and TOC showed that the share of the HA fraction in TOC was lower in LPG treatment than in other treatments (Fig. 2G). The FA fraction showed lower values in PG-amended soil, while in the other treatments, the values were similar (Fig. 2H). On the other hand, the largest share of the HU fraction occurred in LPG treatment, followed by PG and L (Fig. 2I). Control treatment presented the lowest contribution of the HU fraction in TOC. Aboveground dry matter (ADM) production of maize and ruzigrass increased in amended soils (Fig. S1). Maize ADM increased as follows: LPG > L > PG > control (Fig. S1A); ruzigrass produced the same amount of ADM when L ang LPG was applied, both higher than that obtained in control and PG treatments, which did not differ from each other (Fig. S1B).

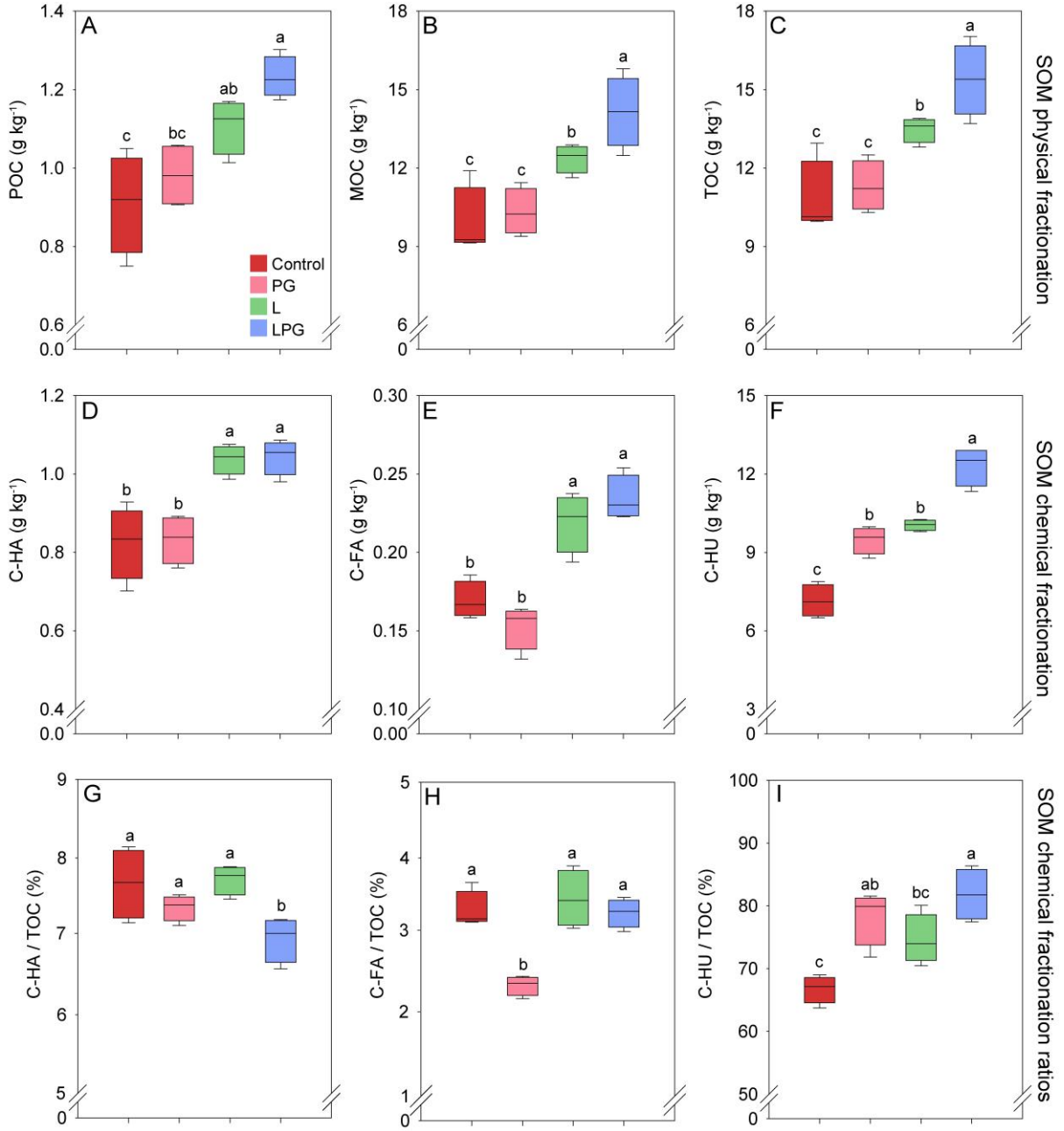


Figure 2. Soil organic carbon concentration from physic fractions (A) particulate organic carbon (POC), (B) mineral-associated organic carbon (MOC), (C) total organic carbon (TOC), and from chemical fractions (D) humic acid (C-HA), (E) fulvic acids (C-FA), (F) humin (C-HU), C-HA/TOC ratio (G), C-FA/TOC ratio (H), and C-HU/TOC ratio (I) of the long-term soil with amendments (lime; PG, phosphogypsum; LPG, lime + phosphogypsum) applied in soil surface.

3.3.3 Impact of Ca-based soil amendments on the soil microbiome

Sensitivity analysis was applied to determine the relative impacts of each treatment on different groups of dependent variables: bacterial and fungal

communities, SOM physical fractionation (SOM-PF), SOM chemical fractionation (SOM-CF), soil fertility and soil physical characteristics (Fig. 3A). In general, the treatments had the greatest impact on soil fertility, followed by the microbiome (bacteria and fungi), SOM-CF, soil physical characteristics, and SOM-PF. In addition, among all groups, the sensitivity values were highest in the control and LPG treatments. We also constructed a principal coordinate analysis (PCA) integrating the regression coefficients from all of the variable groups to identify similarities between the treatments in shifts in soil physicochemical parameters and the microbiome (Fig. 3B). Our analysis indicated a clear segregation of the treatments in the first two axes (83% of the total variation). L- and LPG-amended soils clustered together, whereas PG-amended and control soils were positioned far from each other and from the center of the PCA. In terms of distance from the control, we observed higher proximity for PG than for L and LPG. The control and LPG showed similar levels of sensitivity (Fig. 3A), but L and LPG exhibited high similarity in the PCA analysis (Fig. 3B), most likely because they produced similar effects on the groups of variables corresponding to SOM fractions and soil physicochemical attributes (Fig. 2 and Table 1).

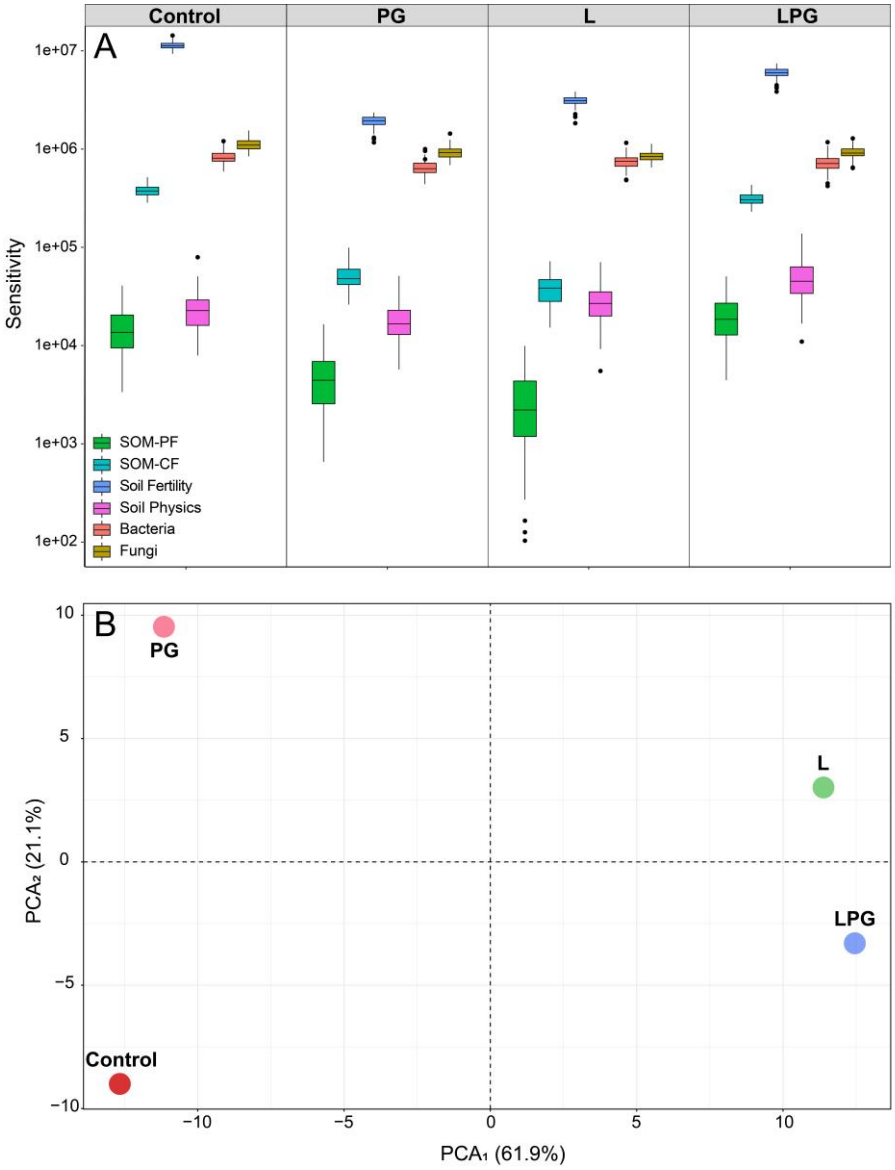


Figure 3. Sensitivity analysis (A), and PCA of regression coefficients (B) of bacterial and fungal amplicon sequence variant (ASVs), SOM physical (SOM-PF) and chemical (SOM-CF) fractions, soil fertility, and soil physics to the environment, according to the soil amendments (B). Control (C), phosphogypsum (PG), lime (L), lime + phosphogypsum (LPG).

For soil fertility, the differences in regression coefficients were greatest between the control and LPG treatments for Ca^{2+} and Mg^{2+} (Fig. 4). In PG-amended soil, Ca^{2+} increased, but Mg^{2+} decreased; in L-amended soil, both Ca^{2+} and Mg^{2+} increased. Similarly, we observed contrasting shifts between the control and LPG treatments in P, NH_4^+, pH, S-SO_4^{2-}, NO_3^- and K^+. Interestingly, the opposite pattern was observed for micronutrients and Al^{3+}. Between the control and LPG treatments, the greatest shifts occurred in Al^{3+}, Fe, Mn, Cu, and Zn.

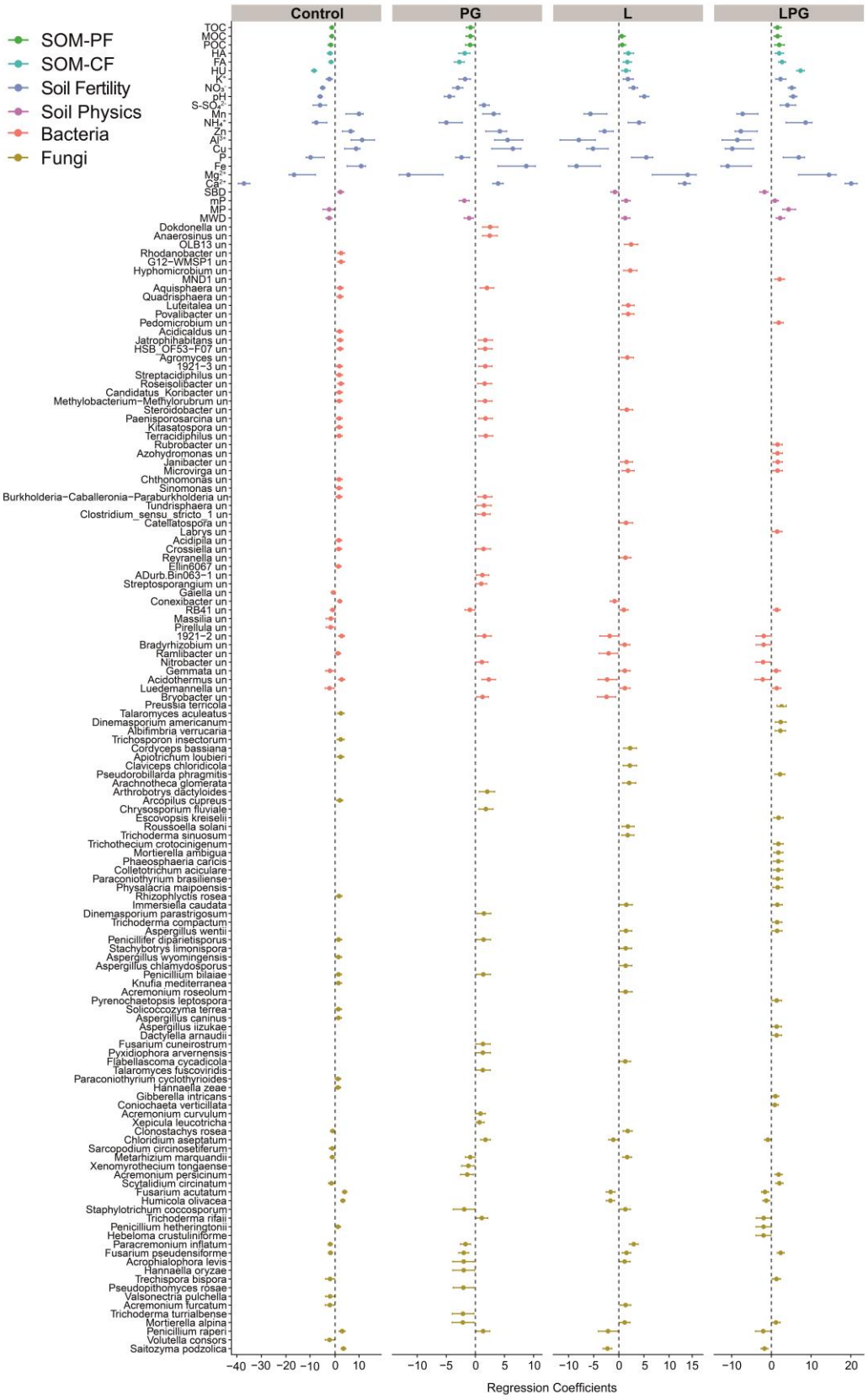


Figure 4. Regression coefficients of only the significant responses of soil factors (physical and chemical fractions, soil fertility, and soil physics), bacterial and fungal communities according to the soil amendments. Coefficients are significant when their 95% confidence interval do not overlap with 0. Unclassified (un).

Within the SOM fractions, the changes in the chemical fractions (HA, FA and HU) were greater than those in the physical fractions (POC, MOC and TOC). Here again, contrasting effects of the control and LPG treatments were observed for HU, FA, HA, POC, MOC, and TOC. For soil physical characteristics, the regression coefficients for the MP and MWD of soil aggregates increased in L- and, most notably, LPG-amended soils. In these same treatments, SBD decreased. Between the control and LPG treatments, the following shifts were obtained: MWD, SBD, MP and mP.

The relative abundances of the microbes also responded to long-term soil amendment. Overall, the treatments influenced the relative abundances of 55 different genera of bacteria (Table S4 and S5). More than half of these bacterial genera (61.8%) belonged to the phyla Proteobacteria (19 genera; 34.5%) and Actinobacteria (15 genera; 27.3%); intermediate responses of Acidobacteriota (6 genera; 10.9%), Chloroflexi (5 genera; 9.1%), Planctomycetota (4 genera; 7.3%), and Firmicutes (3 genera; 5.5%) were observed. Finally, in each of the phyla Armatimonadota, Gemmatimonadota and Verrucomicrobiota, only one bacterial genus (1.8%) was affected by the treatments. The relative abundances of 25 bacterial genera increased and 6 genera decreased in the control. Significant changes were observed in 21 genera in PG (20 positive shifts and 1 negative shift), 19 in L (14 positive shifts and 5 negative shifts), and 14 in LPG (10 positive shifts and 4 negative shifts). Apart from the phylum Proteobacteria, which accounted for the largest number of affected genera considering all treatments (19 genera), the same number of genera were affected in the control and LPG treatments (7 genera, 36.8%). Among the remaining phyla, the largest number of genera affected by the control treatment belonged to the phylum Actinobacteria (10 genera; 66.7%), followed by the phyla Acidobacteriota (4 genera, 66.7%), Chloroflexi (4 genera, 80%) and Planctomycetota (3 genera; 75%). In LPG-amended soil, the same dominant phyla were affected (mostly positively), but the numbers of represented genera were smaller (Actinobacteria = 4 genera, 26.7%; Acidobacteriota = 1 genus, 16.7%; Chloroflexi = 1 genus, 20%; Planctomycetota = 1 genus, 25%). The different Ca-based soil amendments also had unique impacts on the soil microbiome. Two phyla, Firmicutes and Gemmatimonadota, were significantly affected only by PG and the control. The control treatment positively affected one genus of Firmicutes, whereas PG positively affected the relative abundances of 3 different genera of Firmicutes. Within Gemmatimonadota, only one bacterial genus showed significant changes as a result of the two treatments. The phylum

Armatimonadota was detected only in the control treatment (represented by one genus). Likewise, the phylum Verrucomicrobiota was exclusive to the PG treatment.

Compared with bacteria, more fungal representatives were affected by the treatments. Overall, the treatments influenced the relative abundances of 57 genera and 75 species of fungi (Tables S4 and S6). Almost all of these fungal species (96%) belonged to the phyla Ascomycota (63 species; 84%) and Basidiomycota (9 species; 12%). The phyla Mortierellomycota (2 species; 2.7%) and Chytridiomycota (1 species; 1.3%) exhibited the smallest changes in abundance in our study. Interestingly, the LPG treatment had the strongest impact on the fungal community. In LPG-amended soil, the abundances of 24 species of fungi increased, and the relative abundances of 8 other fungal species decreased. The control treatment had the next largest impact, with changes in 28 fungal species (18 positive shifts and 10 negative shifts), followed by the PG and L treatments, which exhibited changes in abundance of 24 species each (PG = 13 positive shifts and 11 negative shifts; L = 19 positive shifts and 5 negative shifts). LPG amendment mainly affected the phyla Ascomycota (26 species) and Mortierellomycota (2 species). The phylum Chytridiomycota was found only in the control treatment.

Based on the regression coefficients, the cluster analysis highlighted groups of variables with similar responses to the treatments, thus allowing us to identify which microbes followed the shifts in soil physicochemical properties (Fig. 5). First, we observed that the Ca-based soil amendments strongly affected most soil fertility variables and HU, clearly segregating them into distinct clusters (C1a-c), in agreement with the sensitivity analysis (Fig. 3A). In addition, we found clustering of SOM physical fractions (POC, MOC and TOC) with many microbes (especially fungi) in the C5 cluster and clustering of SOM chemical fractions (FA and HA), MWD of soil aggregates and K^+ in the C3 cluster, suggesting positive associations between the microbes in these clusters and these soil properties. Although there was no clear segregation between bacteria and fungi, the C2 cluster showed a greater proportion of bacteria (59%) than fungi (39%), both of which were associated with SBD. In addition, we observed a higher proportion of fungi than bacteria in the C3 cluster (bacteria = 37%; fungi = 48%) associated with FA, HA, MWD of aggregates, and K^+; the C4 cluster (bacteria = 37%; fungi = 61%) associated with MP; the C5 cluster (bacteria = 19%; fungi = 71) associated with MOC, POC, TOC, and mP; and the C6 cluster (bacteria = 30%; fungi = 70%) without associations with soil factors.

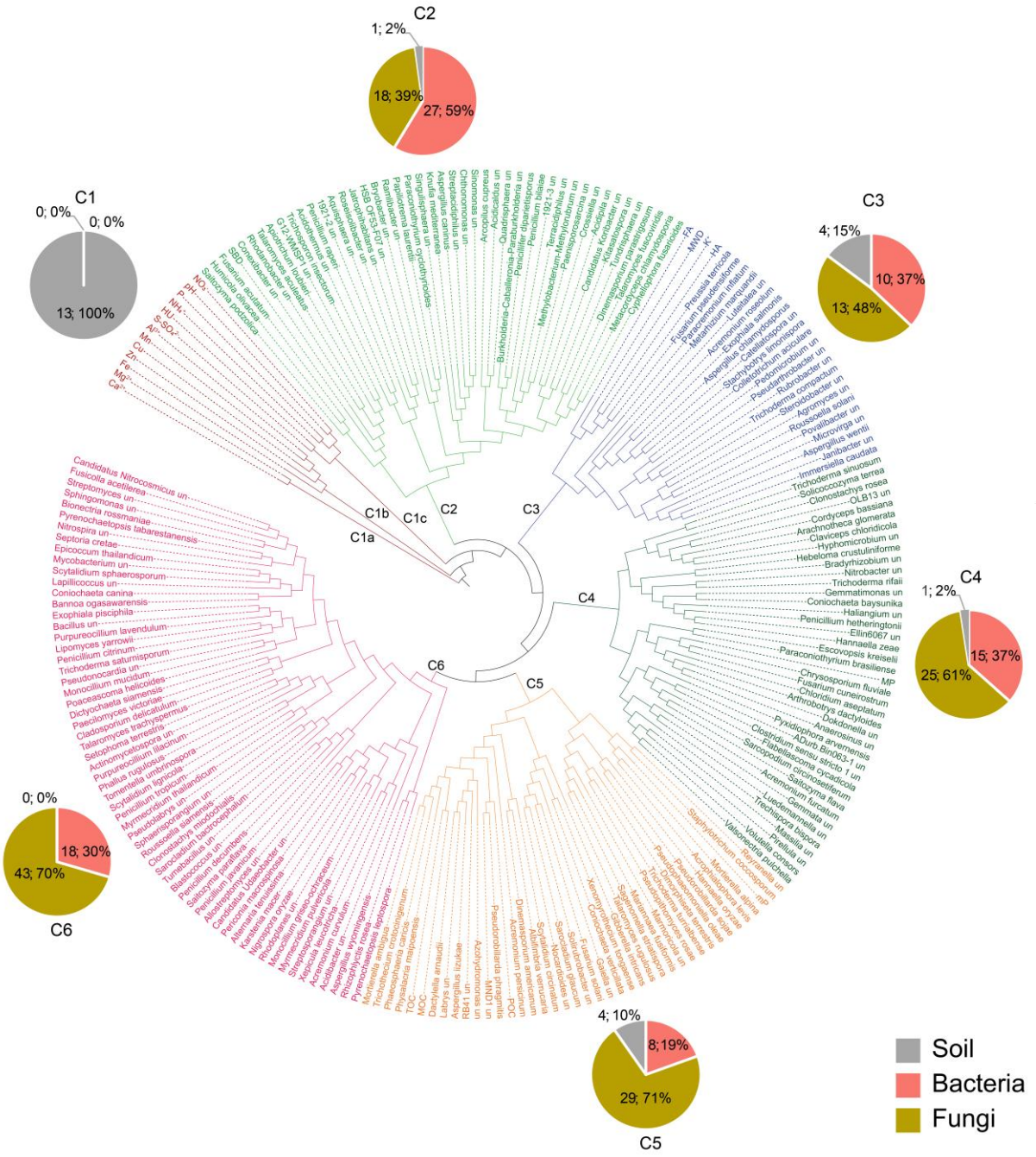


Figure 5. Similarity dendogram between regression coefficients from soil factors, bacterial and fungal communities to the soil amendments. Colors from circular plots represents the variable groups. Particulate organic carbon (POC), mineral- associated organic carbon (MOC), total organic carbon (TOC), humic acid (HA), fulvic acid (FA), humin (HU), mean weight diameter (MWD), soil bulk density (SBD), microporosity (mP), and macroporosity (MP), unclassified (un).

In addition to examining the strengths of shifts in relative abundance in the microbial community, our analysis identified the relevance of the microbes affected by each soil amendment compared with the original soil community (control treatment)

(Fig. 6). Overall, the treatments influenced the relative abundances of 33 fungal species (belonging to 29 genera) and 15 bacterial genera. In PG-amended soil, the relative abundances of 6 microbes increased, among which the abundances of four were originally low in the control treatment (2 bacterial and 2 fungal). PG also reduced the abundances of 10 microbes, all fungal, of which 4 were originally low in abundance and 6 were abundant in the control treatment. In L-amended soil, the abundances of 11 microbes increased (4 bacterial genera and 5 fungal species with low abundance originally and 2 abundant fungal species), whereas 4 other bacterial genera and 6 fungal species that were originally abundant in the control decreased. Finally, LPG amendment increased the abundances of 13 microbes, represented by 2 bacterial genera and 9 fungal species originally with low abundance and 2 abundant fungal species, and decreased the relative abundances of 11 microbes, represented by 1 bacterial genus and 1 fungal species originally with low abundance and 3 bacterial genera and 6 fungal species that were originally abundant in the control treatment. Overall, the different Ca-based soil amendments markedly altered the patterns and relevance of microbial community members in bulk soil. We observed a trend towards reducing the relevance of originally abundant microorganisms and increasing the relevance of low-abundance ones. For example, the relevance of *Acidothermus* and *Aquisphaera* increased in PG, but the relevance of *Acidothermus* decreased over time in the treatments that received L (L alone and LPG). In addition, PG increased the relevance of *Arthrobotrys dactyloides* and *Chrysosporium fluviale*, species with low abundance in the control. Notably, *Mortierella*, which was originally low in abundance, gained relevance in LPG but lost relevance in PG (negative regression coefficient). L- and LPG-amended soils showed similarities in the reduction of the relative abundances of *Acidothermus* and *Chloroflexi 1921-2* bacteria and the fungal species *Saitozyma podzolica*, *Humicola olivacea*, and *Fusarium acutatum*. Interestingly, LPG reduced the relevance of *Nitrobacte*r (originally abundant), *Bradyrhizobium* (bacterial genus originally low in abundance) and *Hebeloma crustuliniforme* (fungus originally low in abundance) but increased the relevance of *MND1* and *Pedomicrobium*, both of which were low-abundance bacteria in the control treatment.

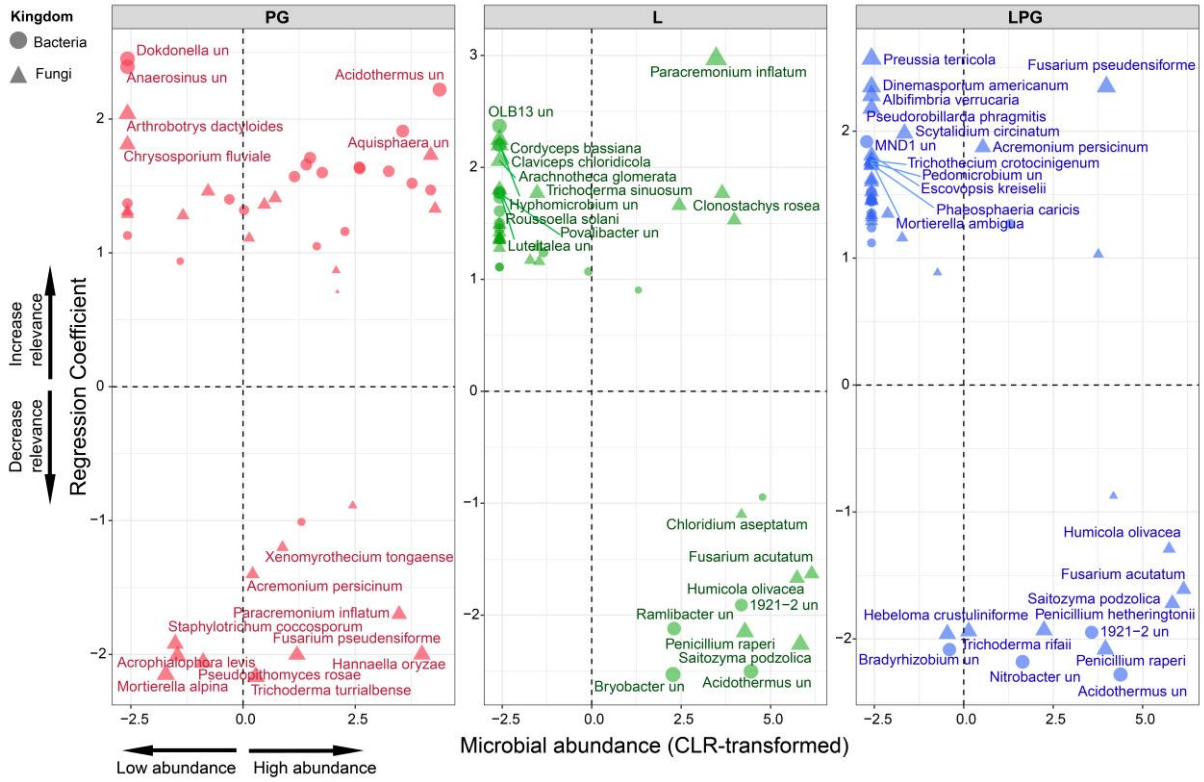


Figure 6. Relationship between the regression coefficient and the abundance (CLR transformed data) of top 30 most negative and most negative shifts from bacterial and fungal taxa in relation to the control treatment. Unclassified (un)

3.4 DISCUSSION

3.4.1 Ca-based soil amendments change soil physicochemical attributes and shape soil chemical and physical carbon fractions

Seventeen years of soil management with amendments in a no-till intercrop system changed the soil physicochemical parameters as well as the amount and quality of SOC. Amendment with L (combined or not with PG) provided the greatest neutralization of soil acidic components (pH and Al^{3+}) and increased the availability of macronutrients (NO_3^-, NH_4^+, P, K^+, Ca^{2+}, Mg^{2+} and S-SO_4^{2-}), whereas LPG amendment efficiently increased N (NO_3^- and NH_4^+), Ca^{2+} and S-SO_4^{2-} availability.

Applying PG alone resulted in a small change in soil physicochemical properties compared with the control treatment. Although PG is not considered an acid-neutralizing compound (Zoca and Penn, 2017; Bossolani et al., 2018), combining L and PG synergistically exploits L's ability to correct soil acidity and PG's ability to

promote Al^{3+} neutralization and increase Ca^{2+} input in the soil. Such effects are particularly relevant under the conditions of low Ca^{2+} availability found in highly weathered tropical soils (Carmeis Filho et al., 2017b; Crusciol et al., 2019; Bossolani et al., 2020a). High L inputs to the soil surface can drastically reduce the availability of soil micronutrients (Fe, Mn, Cu and Zn) by increasing pH (Pegoraro et al., 2006; Dhaliwal et al., 2019), as observed in our study, and thus PG is an efficient amendment that provides Ca^{2+} to the soil without further changing the soil pH and reducing crop yield (Costa and Crusciol, 2016; Bossolani et al., 2020a).

The primary effects of L on soil fertility trigger cascading effects on soil quality and crop responses. We showed increases of grain and biomass productivity (in the form of aboveground residues and belowground root biomass) in the current study as well as in our previously studies, in the same experimental area (Carmeis Filho et al., 2017b; Bossolani et al., 2020a) by L alone or in combination with PG. These improvements are positively correlated with increases in SOM. We observed increases in SOM fractions in soils amended with L or, most notably, LPG. Biomass-C input by aboveground dry matter production and high Ca^{2+} availability in amended soils are important for increasing C concentrations in the soil and also indicate a greater capacity for atmospheric C sequestration in fertile systems managed with Ca-based soil amendments (Inagaki et al., 2016; Carmeis Filho et al., 2017a).

Higher biomass production from crop residues in amended soils is the main source of C contributing to increased SOC concentrations (Inagaki et al., 2016; Carmeis Filho et al., 2017b), as observed in our results. Additionally, Ca^{2+} availability is an important ionic bridge between clay particles and SOM, resulting in greater soil aggregation and consequent C protection (Briedis et al., 2012; Aye et al., 2016). Thus, Ca^{2+} availability has high potential as an indicator of SOC accumulation in NTS (Briedis et al., 2012). Our results demonstrate synergism between L and PG, two Ca-based soil amendments that act via distinct mechanisms to increase nutrient availability without abruptly increasing pH.

The long-term Ca-based soil amendments also affected the physical characteristics of the soil, with the formation of larger stable aggregates (soil aggregate MWD), higher MP, lower SBD and, consequently, improved soil particle structure. The higher Ca^{2+} availability in the L-amended soils (with or without PG) may have played an important role in the formation of stable soil aggregates, as this element is essential for root growth and consequent formation of colloids by the mechanical action of roots

and the release of root exudates (Carmeis Filho et al., 2018). Additionally, Ca^{2+}, Fe, and Al^{3+} have been described as “persistent soil aggregating agents” that are responsible for binding between SOM and clay particles and the formation of organo-mineral complexes (Tisdall and Oades, 1982; Fornara et al., 2011). As shown here and in our previous work (Carmeis Filho et al., 2017b), these organo-mineral complexes protect SOM-derived C against decomposition processes over time.

Lime has been reported to increase microbial activity and the mineralization of labile organic matter (Inagaki et al., 2016), but these reports were based on studies carried out in agricultural systems managed with soil disturbance operations, such as plowing and harrowing (Aye et al., 2016; Joris et al., 2016), and not the application of L on the surface of tropical soils in long-term no-till crop rotation systems. In addition, studies associating L with higher soil respiration rates and thus consumption of SOM pools by microorganisms (Holland et al., 2018) considered only short-term periods. Under long-term effects of Ca-based soil amendments (particularly Ca-based) in no-till crop systems, the return of biomass-C over time is high (Carmeis Filho et al., 2017b), balancing the system with positive C turnover. During the 17-year period of the present study, only 4 applications of PG, L or LPG were carried out based on the results of soil chemical analyses performed annually as described in the Material and Methods section.

Plant residues and organic compounds in soil are transformed by microbes (Waksman and Gerretsen, 1931; Gregorich et al., 1996; Bending et al., 2002; Hilscher et al., 2009; Kuramae et al., 2013; Suleiman et al., 2019), which together with soil physicochemical quality, strongly influence the stabilization of SOM fractions (Castellano et al., 2015). The fractions of labile organic carbon, i.e., POC, and stable organic carbon, i.e., MOC, were highest in LPG-amended soil, whereas the SOM chemical fractions HA, FA and HU did not differ between soil amended with L alone and with LPG. Our results suggest that the enhancement of SOM mineralization by L and LPG favors the decomposition of non-recalcitrant compounds, the synthesis of more stable molecules and improved SOM quality (Stevenson, 1994). By contrast, the content of easily microbial degradable SOM compounds (POC) in LPG-amended soil can further act as major available nutrients and C sources for microbial metabolic activity. Interestingly, PG increases the share of the HU fraction in TOC, however, it reduces FA. LPG-amended soil also increased the HU fraction. In both treatments, there is a considerable addition of Ca^{2+}, which favor in the process of stabilizing SOM

and reducing the biochemical evolution of young organic compounds (Tisdall and Oades, 1982; Fornara et al., 2011).

3.4.2 Linking the soil microbiome with the legacy of Ca-based soil amendments for soil quality

The vast majority of studies of agricultural management practices are limited to examining single factors such as soil tillage (Fuentes et al., 2006; Babin et al., 2019), fertilizers (Dai et al., 2018; Babin et al., 2019; Tian et al., 2019), amendments (Liu et al., 2018; Guo et al., 2019; Pang et al., 2019) or the behavior of select groups of bacteria or fungi. By contrast, our study integrated generalized joint attribute modeling (GJAM) and data on SOM quality (SOM physical and chemical fractions), soil chemical attributes (soil fertility), soil physical attributes (soil physics) and the soil microbiome (bacterial and fungal communities). This study is the first to investigate how the surface application of Ca-based soil amendments (acidity corrective and/or soil conditioner) in a tropical no-till intercrop system over 17 years influences soil physicochemical properties and bacterial and fungal communities. By going beyond isolated effects of the Ca-based soil amendments on each of the soil attributes and the microbiome, our joint data analysis provides strong evidence on how Ca-based soil amendments shift soil factors that modulate the microbiome, which in turn plays a crucial role in shaping the chemical fractions of SOM, soil physical characteristics and soil physical fractions to ultimately increase soil quality.

The sensitivity analysis revealed how each group of variables was affected by the treatments. The amendments strongly affected soil parameters, especially soil fertility, and LPG had the greatest impact, consistent with the results of the soil physicochemical analysis and the regression coefficients in GJAM. Our control treatment represented 17 years of standard agriculture with fertilizers and biomass-C inputs in all cropping seasons but without addition of L and PG amendments. This agricultural model has its own effects on soil chemical parameters and leads to strong acidification and nutrient displacement (e.g., N, P, Ca^{2+} and Mg^{2+}) in the weathered soil of this tropical region (Fageria and Baligar, 2008).

LPG amendment strongly impacted the soil C concentration and nutrient availability over the 17 years of the experiment. Interestingly, the sensitivity analysis showed that the responses of the bacterial and fungal communities were similar among

the treatments. After soil fertility, microbes were the group of variables most strongly affected by the amendments. This result demonstrates that long-term agriculture without management measures improving soil quality (i.e., without Ca-based soil amendments) impacts the soil microbiome, selecting specific microorganisms and consequently impacting the soil ecosystem services delivered by microbes. In addition, PCA confirmed that the changes in the soil microbiome over the 17-year period were greatest in the control and LPG treatments and that the treatments that received L (as L or LPG) were most similar in terms of changes in soil physicochemical parameters and the soil microbiome. Despite the similar levels of impact (sensitivity) on the microbiome among the treatments, distinct groups of microorganisms were selected in each treatment, demonstrating the capacity of each soil amendment to modulate and rearrange distinct soil microbial communities compared to the control.

The low-fertility soils in the control and PG treatments favored the largest number of members within the phyla Actinobacteria (i.e., *Acidothermus*, *Crossiella* and, *Jatrophihabitans*), Acidobacteriota (i.e., *Terracidiphilus* and *Acidipila*) and Chloroflexi. These bacterial genera are known to be oligotrophic, with high resistance to adverse conditions (Rodrigues et al., 2013), such as acidic environments with low nutrient availability (Kielak et al., 2016; Kuramae and Costa, 2019) and the presence of heavy metals (Hemmat-Jou et al., 2018; Wang et al., 2018). These results are in accordance with the analyses of the soil in the control and PG treatments, that had the lowest pH values and nutrient availability (e.g., N, P, Ca^{2+} and Mg^{2+}) and highest values of Al^{3+}, Fe, Mn, Cu and Zn.

We also observed positive shifts in the relative abundances of several genera of the phylum Proteobacteria in all treatments, although the specific genera differed in each treatment. In the control, these genera included *Acidicaldus* and *Rhodanobacter*, which are characteristic of acidic and oligotrophic environments and play a role in the cycling of metals such as Fe (Hedrich et al., 2011), and other genera related to nitrification (i.e., *Ellin6067*) (Ye et al., 2016). Interestingly, the genus *Methylobacterium-Methylorubrum*, which was previously described as a plant-associated microorganism with beneficial roles in plant growth via changes in auxin and cytokinin balance (Abanda-Nkpwatt et al., 2006), was recently associated with parasitic potential rather than benefits in soybean plants, as it competes for nitrogen compounds (allantoin and allantoic acid) from biological nitrogen fixation (BNF) as a N source (Minami et al., 2016). At our study site, maize is intercropped with ruzigrass

and soybean in crop rotation, and therefore the increase of this microorganism may have negative effects on soybean performance; further studies are needed to confirm this hypothesis.

In LPG-amended soil, interesting positive shifts were observed in the relative abundances of *Microvirga*, *Labrys* and *Pedomicrobium*, organisms that are notably copiotrophic, while negative shifts were observed in *Bradyrhizobium* and *Nitrobacter*, commonly denoted as oligotrophic (Msaddak et al., 2017; Yao et al., 2017; Moretti et al., 2018). These changes can be partially explained by the soil trophic level (Holland et al., 2018). Agricultural management with Ca-based soil amendments increases the availability of C and soil nutrients in three main ways: i) increased soil pH results in an increase in negatively loaded bridges in colloids, thereby preventing nutrient displacement, ii) direct input of nutrients from the Ca-based soil amendments; and iii) increased biomass-C yield from crop residues and nutrient cycling over time (Carmeis Filho et al., 2017b). Thus, Ca-based soil amendments reduce the selective pressure of nutrient availability on microorganisms (Dai et al., 2018; Leff et al., 2015), as observed for *Bradyrhizobium* and *Nitrobacter* in LPG-amended soil. The decomposition and recycling of organic matter is largely carried out by soil microorganisms capable of converting organic components into nutrients available to plants (Steinberger and Shore, 2009). In our previous work at the same site, we observed an increase in the abundance of the *nifH* gene in soils amended with both L and PG (Bossolani et al., 2020a), and therefore greater relevance of *Bradyrhizobium* in this treatment was expected. Instead, reduced relevance of *Bradyrhizobium* was observed in LPG-amended soil, suggesting that the *nifH* gene found previously may be derived from microorganisms other than *Bradyrhizobium*. Future studies should sequence specific regions of DNA related to biological nitrogen fixation (BNF) to further elaborate this finding. In addition, fertile soils increase plants' capacity to compete with microorganisms for soil resources, thereby reducing available substrates for microbial growth and partially explaining the reduction of the role of *Nitrobacter* in LPG-amended soil. Another explanation is that nitrification is lower in intercropping systems with grain crops and tropical forage grasses (Coskun et al., 2017; Bossolani et al., 2020a) like the one in the present study. Subbarao et al. (2015) suggested that forage grasses release a compound known as **braquialactone that is capable of** suppressing nitrification.

Interestingly, the changes in soil fertility due to LPG amendment fostered increases in a wide range of saprophytic fungi (soil, wood, and plant saprotrophs) that decompose organic matter (i.e., *Preussia terricola*, *Albifimbria verrucaria*, *Phaeosphaeria caricis*, *Escovopsis kreiselii* and *Scytalidium circinatum*). The activity of microorganisms on the soil surface is high due to the deposition of crop residues (Córdova et al., 2018). Our results showed that the long-term application of LPG on weathered tropical soils promoted fungal groups that increase C mineralization potential, thereby favoring the decomposition of non-recalcitrant compounds into stable C molecules and improving SOM quality. Contrary to many studies that have reported reduced fungal activity in soils with higher pH (Bothe, 2015; Holland et al., 2018), we observed an increase in the participation of these microorganisms in soils amended with L and, in particular, LPG. The positive relationship between the abundance of these fungi and the amendments might be explained by the high SOC in these treatments obtained by the long-term no-till cropping system. Furthermore, remarkably, the increase in fungi was also related to the formation of larger aggregates (higher MWD), which made LPG-amended soil less dense (lower SBD values) and more porous (higher MP). Additionally, fungi had a greater role in aggregate formation than bacteria, corroborating the results of Bossuyt et al. (2001) for a different soil type and conditions. Fungi can produce metabolites (e.g., polysaccharides, glomalin and lipids) that increase aggregate formation (Mukerji et al., 2006).

The locations of bacteria and fungi within soil pores are critical determinants of the survival and activity of these microorganisms; fungi have a preference for growing in macroaggregates, while bacteria prefer small pores (Ding et al., 2015). Our results showed greater shifts in bacteria in the control and PG-amended soils but greater shifts in fungi in L- and LPG-amended soils. Accordingly, the control and PG-amended soils had the highest SBD, whereas the L- and LPG-amended soils had the highest MP.

Finally, we observed changes in the relevance of specific microorganisms in each soil amendment compared with the control. Overall, the Ca-based soil amendments reduced the relevance of abundant microorganisms (bacteria and fungi) and increased the relevance of low-abundance microorganisms. Interestingly, L and, in particular, LPG amendment increased the relevance of several microorganisms (mostly fungi) that were not abundant in the control treatment, suggesting an increase in the ecological role of low-abundance microorganisms. As expected, the nitrogen fixer *Bradyrhizobium* and the ectomycorrhizal fungus *Hebeloma crustuliniforme*, both

of which were naturally low in abundance in the control, became even less relevant in LPG-amended soil, probably due to the greater availability of nutrients in this treatment. Similarly, *Nitrobacter*, an abundant genus in the control, lost relevance in LPG-amended soil, suggesting reduced nitrification as observed previously by Bossolani et al. (2020a). With regard to fungi, increasing the relevance of less-abundant species can benefit the agricultural system, such as *Arthrobotrys dactyloides*, a nematode-trapping fungus (Strom et al., 2019, 2020), in PG-amended soil and *Mortierella ambigua*, an opportunistic microbe of arable soils with N-rich organic residues in the early stages of decomposition (De Tender et al., 2019) that can parasitize nematode eggs (Lupatini et al., 2019), in LPG-amended soil. Interestingly, the bacteria genera *Pedomicrobium* and Proteobacteria *MND1*, both of which were low in abundance in the control but had greater relevance in the LPG treatment, have been reported to use hydrocarbon contaminants as a C source to oxidize and retain Fe and Mn in soil environments (Orcutt et al., 2011). In addition, Yao et al. (2017) found strong correlations of *Pedomicrobium* with pH, TOC and N in long-term biochar-amended soils. The enrichment of low-abundance microorganisms by Ca-based soil amendments can be beneficial for agricultural systems by promoting beneficial bacteria and fungi that increase soil functionality (De Tender et al., 2019; Lupatini et al., 2019; Suleiman et al., 2019). Most previous studies have focused on dominant taxa and minimized the importance of low-abundance taxa. However, recent studies have reported that these low-abundance taxa are major determinants of soil multifunctionality (Jiao et al., 2019; Wagg et al., 2019; Zhu et al., 2020). Low-abundance bacteria and fungi are now recognized as drivers of key functions in agroecosystems that serve as an adverse pool to enhance both resilience and resistance under environmental disturbances (Jiao et al., 2019). Under favorable conditions, these microorganisms can eventually play important roles and provide unique functional traits (Jiao et al., 2019; Wagg et al., 2019). Thus, low-abundance microbial taxa could be a genetic reservoir that is activated under appropriate conditions for growth.

3.5 CONCLUSIONS

Our findings suggest that, in a long-term no-till intercropping system, amendments that neutralize acidity of the soil play a crucial role in increasing C

concentrations (labile and stable fractions) and soil physicochemical attributes, especially considering the synergism between L and PG (LPG amendment). Our model showed that LPG amendment increased soil fertility by promoting changes in the soil microbiome to improve SOM fractions and soil physical characteristics. The effects of L and LPG on the fungal community resulted in similar changes in SOM fractions (physical and chemical), suggesting important roles of these fungi in the transformation of SOM compounds. Finally, we observed that over 17 years, the Ca-based soil amendments (especially LPG) increased the relevance of low-abundance microorganisms and reduced the relevance of other microorganisms that were originally abundant compared with the control treatment, suggesting an increased role of these low-abundance microorganisms in ecosystem functioning in these soils.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by São Paulo Research Foundation (FAPESP) (Grant 2018/11063-7 and 2019/12764-1), and The Netherlands Organization for Scientific Research (NWO) grant 870.15.022. In addition, the second author would like to thank the National Council for Scientific and Technological Development (CNPq) for an award for excellence in research. Publication number 7163 of the Netherlands Institute of Ecology (NIOO-KNAW).

REFERENCES

Abanda-Nkpwatt, D., Müsch, M., Tschiersch, J., Boettner, M., Schwab, W., 2006. Molecular interaction between Methylobacterium extorquens and seedlings: Growth promotion, methanol consumption, and localization of the methanol emission site. Journal of Experimental Botany 57, 4025–4032. doi:10.1093/jxb/erl173

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22, 711–728. doi:https://doi.org/10.1127/0941-2948/2013/0507

Aye, N.S., Sale, P.W.G., Tang, C., 2016. The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils. Biology and Fertility of Soils 52, 697–709. doi:10.1007/s00374-016-1111-y

Babin, D., Deubel, A., Jacquiod, S., Sørensen, S.J., Geistlinger, J., Grosch, R., Smalla, K., 2019. Impact of long-term agricultural management practices on soil prokaryotic communities. Soil Biology & Biochemistry 129, 17–28. doi:10.1016/j.soilbio.2018.11.002

Bardsley, C.E., Lancaster, J.D., 1960. Determination of reserve sulfur and soluble sulfates in soils. Soil Science Society of America Journal 24, 265–268. doi:https://doi.org/10.2136/sssaj1960.03615995002400040015x

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biology & Biochemistry 34, 1073–1082.

Bian, M., Zhou, M., Sun, D., Li, C., 2013. Molecular approaches unravel the mechanism of acid soil tolerance in plants. Crop Journal 1, 91–104. doi:10.1016/j.cj.2013.08.002

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020a. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375, 114476. doi:10.1016/j.geoderma.2020.114476

Bossolani, J.W., dos Santos, F.L., Meneghette, H.H.A., Sanches, I.R., Moretti, L.G., Parra, L.F., Lazarini, E., 2020b. Soybean in Crop Rotation with Maize and Palisade Grass Intercropping Enhances the Long-term Effects of Surface Liming in No-till System. Journal of Soil Science and Plant Nutrition 20, 1–12. doi:10.1007/s42729-020-00347-2

Bossolani, J.W., Lazarini, E., dos Santos, F.L., Sanches, I.R., Meneghette, H.H.A., Parra, L.F., Souza, L.G.M., 2018. Surface reapplication of lime and gypsum on maize cultivated sole and intercropped with Urochloa. Communications in Soil Science and Plant Analysis, 49(15), 1855-1868. doi: 10.1080/00103624.2018.1475565

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195–208. doi:10.1016/S0929-1393(00)00116-5

Bothe, H., 2015. The lime-silicate question. Soil Biology & Biochemistry 89, 172–183. doi:10.1016/j.soilbio.2015.07.004

Bowman, W.D., Cleveland, C.C., Halada, Ĺ., Hreško, J., Baron, J.S., 2008. Negative impact of nitrogen deposition on soil buffering capacity. Nature Geoscience 1, 767–770. doi:10.1038/ngeo339

Briedis, C., Sá, J.C. de M., Caires, E.F., Navarro, J. de F., Inagaki, T.M., Boer, A., Neto, C.Q., Ferreira, A. de O., Canalli, L.B., Santos, J.B. dos, 2012. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. Geoderma 170, 80–88. doi:10.1016/j.geoderma.2011.10.011

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Journal 11, 2639–2643. doi:10.1038/ismej.2017.119

Cambardella, C.A., Elliot, E.T., 1994. Carbon and nitrogen dynamics of soil organic matter size/density fractions under different management practices. Soil Science Society of America Journal 57, 1071–1076. doi:https://doi.org/10.2136/sssaj1994.03615995005800010017x

Carmeis Filho, A.C.A., Crusciol, C.A.C., Guimarães, T.M., Calonego, J.C., da Costa, C.H.M., 2018. Changes in Soil Physical Properties and Carbon Protection Mechanisms by Surface Application of Lime in a Tropical No-Tillage System. Soil Science Society of America Journal 82, 56–65. doi:10.2136/sssaj2017.04.0120

Carmeis Filho, A.C.A., Crusciol, C.A.C., Guimarães, T.M., Calonego, J.C., Mooney, S.J., 2017a. Impact of amendments on the physical properties of soil under tropical long-term no till conditions. PLoS ONE 12, 27959897. doi:10.1371/journal.pone.0173011

Carmeis Filho, A.C.A., Penn, C.J., Crusciol, C.A.C., Calonego, J.C., 2017b. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. Agriculture, Ecosystems and Environment 241, 11–23. doi:10.1016/j.agee.2017.02.027

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., Six, J., 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology 21, 3200–3209. doi:10.1111/gcb.12982

Ciavatta, C., Govi, M., Antisari, L.V., Sequi, P., 1990. Characterization of humified compounds by extraction and fractionation on solid polyvinylpyrrolidone. Journal of Chromatography A 509, 141–146. doi:10.1016/S0021-9673(01)93248-0

Clark, J.S., Nemergut, D., Seyednasrollah, B., Turner, P.J., Zhang, S., 2017. Generalized joint attribute modeling for biodiversity analysis: Median-zero, multivariate, multifarious data. Ecological Monographs. doi:10.1002/ecm.1241

Córdova, S.C., Olk, D.C., Dietzel, R.N., Mueller, K.E., Archontouilis, S. V., Castellano, M.J., 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology & Biochemistry 125, 115–124. doi:10.1016/j.soilbio.2018.07.010

Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. How Plant Root Exudates Shape the Nitrogen Cycle. Trends in Plant Science 22, 661–673. doi:10.1016/j.tplants.2017.05.004

Costa, C.H.M., Crusciol, C.A.C., 2016. Long-term effects of lime and phosphogypsum application on tropical no-till soybean-oat-sorghum rotation and soil chemical properties. European Journal of Agronomy 74, 119–132. doi:10.1016/j.eja.2015.12.001

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutrient Cycling in Agroecosystems 115, 347–372. doi:10.1007/s10705-019-10017-0

Cusser, S., Bahlai, C., Swinton, S.M., Robertson, G.P., Haddad, N.M., 2020. Long-term research avoids spurious and misleading trends in sustainability attributes of no-till. Global Change Biology 26, 3715–3725. doi:10.1111/gcb.15080

Dai, Z., Su, W., Chen, H., Barberán, A., Zhao, H., Yu, M., Yu, L., Brookes, P.C., Schadt, C.W., Chang, S.X., Xu, J., 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. Global Change Biology 24, 3452–3461. doi:10.1111/gcb.14163

De Tender, C., Mesuere, B., Van der Jeugt, F., Haegeman, A., Ruttink, T., Vandecasteele, B., Dawyndt, P., Debode, J., Kuramae, E.E., 2019. Peat substrate amended with chitin modulates the N-cycle, siderophore and chitinase responses in the lettuce rhizobiome. Scientific Reports 9, 1–11. doi:10.1038/s41598-019-46106-x

Dhaliwal, S.S., Naresh, R.K., Mandal, A., Singh, R., Dhaliwal, M.K., 2019. Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. Environmental and Sustainability Indicators 1–2, 100007. doi:10.1016/j.indic.2019.100007

Ding, X., Liang, C., Zhang, B., Yuan, Y., Han, X., 2015. Higher rates of manure application lead to greater accumulation of both fungal and bacterial residues in macroaggregates of a clay soil. Soil Biology & Biochemistry 84, 137–146. doi:10.1016/j.soilbio.2015.02.015

Evans, C.D., Jones, T.G., Burden, A., Ostle, N., Zieliński, P., Cooper, M.D.A., Peacock, M., Clark, J.M., Oulehle, F., Cooper, D., Freeman, C., 2012. Acidity controls on dissolved organic carbon mobility in organic soils. Global Change Biology 18, 3317–3331. doi:10.1111/j.1365-2486.2012.02794.x

Fageria, N.K., Baligar, V.C., 2008. Chapter 7 Ameliorating Soil Acidity of Tropical Oxisols by Liming For Sustainable Crop Production. Advances in Agronomy 99, 345–399. doi:10.1016/S0065-2113(08)00407-0

Fontoura, S.M.V., de Castro Pias, O.H., Tiecher, T., Cherubin, M.R., de Moraes, R.P., Bayer, C., 2019. Effect of gypsum rates and lime with different reactivity on soil acidity and crop grain yields in a subtropical Oxisol under no-tillage. Soil and Tillage Research 193, 27–41. doi:10.1016/j.still.2019.05.005

Fornara, D.A., Steinbeiss, S., Mcnamara, N.P., Gleixner, G., Oakley, S., Poulton, P.R., Macdonald, A.J., Bardgett, R.D., 2011. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. Global Change Biology 17, 1925–1934. doi:10.1111/j.1365-2486.2010.02328.x

Fuentes, J.P., Bezdicek, D.F., Flury, M., Albrecht, S., Smith, J.L., 2006. Microbial activity affected by lime in a long-term no-till soil. Soil and Tillage Research 88, 123–131. doi:10.1016/j.still.2005.05.001

Gibbs, H.K., Salmon, J.M., 2015. Mapping the world's degraded lands. Applied Geography 57, 12–21. doi:10.1016/j.apgeog.2014.11.024

Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.R., 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. Soil Science 161, 680–693.

Guo, A., Ding, L., Tang, Z., Zhao, Z., Duan, G., 2019a. Microbial response to CaCO 3 application in an acid soil in southern China. Journal of Environmental Sciences (China) 79, 321–329. doi:10.1016/j.jes.2018.12.007

Guo, A., Ding, L., Tang, Z., Zhao, Z., Duan, G., 2019b. Microbial response to CaCO3 application in an acid soil in southern China. Journal of Environmental Sciences 79, 321–329. doi:10.1016/j.jes.2018.12.007

Heanes, D.L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in Soil Science and Plant Analysis 15, 1191–1213. doi:https://doi.org/10.1080/00103628409367551

Hedrich, S., Schlömann, M., Barrie Johnson, D., 2011. The iron-oxidizing proteobacteria. Microbiology 157, 1551–1564. doi:10.1099/mic.0.045344-0

Hemmat-Jou, M.H., Safari-Sinegani, A.A., Mirzaie-Asl, A., Tahmourespour, A., 2018. Analysis of microbial communities in heavy metals-contaminated soils using the metagenomic approach. Ecotoxicology 27, 1281–1291. doi:10.1007/s10646-018-1981-x

Hilscher, A., Heister, K., Siewert, C., Knicker, H., 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemistry 40, 332–342.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Science of the Total Environment 610–611, 316–332. doi:10.1016/j.scitotenv.2017.08.020

Inagaki, T.M., de Moraes Sá, J.C., Caires, E.F., Gonçalves, D.R.P., 2016. Lime and gypsum application increases biological activity, carbon pools, and agronomic productivity in highly weathered soil. Agriculture, Ecosystems and Environment 231, 156–165. doi:10.1016/j.agee.2016.06.034

Jha, N., Palmada, T., Berben, P., Saggar, S., Luo, J., McMillan, A.M.S., 2020. Influence of liming-induced pH changes on nitrous oxide emission, nirS, nirK and nosZ gene abundance from applied cattle urine in allophanic and fluvial grazed pasture soils. Biology and Fertility of Soils 56, 811–824. doi:10.1007/s00374-020-01460-1

Jiao, S., Wang, J., Wei, G., Chen, W., Lu, Y., 2019. Dominant role of abundant rather than rare bacterial taxa in maintaining agro-soil microbiomes under environmental disturbances. Chemosphere 235, 248–259. doi:10.1016/j.chemosphere.2019.06.174

Joris, H.A.W., Caires, E.F., Scharr, D.A., Bini, Â.R., Haliski, A., 2016. Liming in the conversion from degraded pastureland to a no-till cropping system in Southern Brazil. Soil and Tillage Research 162, 68–77. doi:10.1016/j.still.2016.04.009

Kemper, W.D., Chepil, W.S., 1965. Size distribution of aggregates. Methods of Soil Analysis: Part 1 Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling 9, 499–510. doi:https://doi.org/10.2134/agronmonogr9.1.c39

Kielak, A.M., Scheublin, T.R., Mendes, L.W., van Veen, J.A., Kuramae, E.E., 2016. Bacterial community succession in pine-wood decomposition. Frontiers in Microbiology 7, 1–12. doi:10.3389/fmicb.2016.00231

Klute, A., 1986. Water retention: Laboratory methods, in: Methods of Soil Analysis. Part 1- Physical and Mineralogical Methods. SSSA, Madison, WI, pp. 635–662.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22, 5271–5277. doi:10.1111/mec.12481

Kuramae, E.E., Costa, O.Y. de A., 2019. Acidobacteria. Encyclopedia of Microbiology (Fourth Edition) 1–8. doi:https://doi.org/10.1016/B978-0-12-809633-8.20780-2

Kuramae, E.E., Gamper, H.A., Yergeau, E., Piceno, Y.M., Brodie, E.L., Desantis, T.Z., Andersen, G.L., Van Veen, J.A., Kowalchuk, G.A., 2010. Microbial secondary succession in a chronosequence of chalk grasslands. ISME Journal 4, 711–715. doi:10.1038/ismej.2010.11

Kuramae, E.E., Hillekens, R.H.E., de Hollander, M., van der Heijden, M.G.A., van den Berg, M., van Straalen, N.M., Kowalchuk, G.A., 2013. Structural and functional variation in soil fungal communities associated with litter bags containing maize leaf. FEMS Microbiology Ecology 84, 519–531. doi:10.1111/1574-6941.12080

Kuramae, E.E., Yergeau, E., Wong, L.C., Pijl, A.S., Van Veen, J.A., Kowalchuk, G.A., 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiology Ecology 79, 12–24. doi:10.1111/j.1574-6941.2011.01192.x

Kušliene, G., Rasmussen, J., Kuzyakov, Y., Eriksen, J., 2014. Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by 13C and 15N labelled exudates. Soil Biology & Biochemistry 76, 22–33. doi:10.1016/j.soilbio.2014.05.003

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America 112, 10967–10972. doi:10.1073/pnas.1508382112

Leite, M.F.A., Kuramae, E.E., 2020. You must choose, but choose wisely: Model-based approaches for microbial community analysis. Soil Biology & Biochemistry 151. doi:10.1016/j.soilbio.2020.108042

Liu, J., Liu, M., Wu, M., Jiang, C., Chen, X., Cai, Z., Wang, B., Zhang, J., Zhang, T., Li, Z., 2018. Soil pH rather than nutrients drive changes in microbial community

following long-term fertilization in acidic Ultisols of southern China. Journal of Soils and Sediments 18, 1853–1864. doi:10.1007/s11368-018-1934-2

Liu, X.Y., Rezaei Rashti, M., Esfandbod, M., Powell, B., Chen, C.R., 2018. Liming improves soil microbial growth, but trash blanket placement increases labile carbon and nitrogen availability in a sugarcane soil of subtropical Australia. Soil Research 56, 235–243. doi:10.1071/SR17116

Lupatini, M., Korthals, G.W., Roesch, L.F.W., Kuramae, E.E., 2019. Long-term farming systems modulate multi-trophic responses. Science of the Total Environment 646, 480–490. doi:10.1016/j.scitotenv.2018.07.323

Meng, C., Tian, D., Zeng, H., Li, Z., Yi, C., Niu, S., 2019. Global soil acidification impacts on belowground processes. Environmental Research Letters 14, 074003. doi:10.1088/1748-9326/ab239c

Minami, T., Anda, M., Mitsui, H., Sugawara, M., Kaneko, T., Sato, S., Ikeda, S., Okubo, T., Tsurumaru, H., Minamisawa, K., 2016. Metagenomic analysis revealed methylamine and Ureide utilization of soybean-associated Methylobacterium. Microbes and Environments 31, 268–278. doi:10.1264/jsme2.ME16035

Moretti, L.G., Lazarini, E., Bossolani, J.W., Parente, T.L., Caioni, S., Araujo, R.S., Hungria, M., 2018. Can additional inoculations increase soybean nodulation and grain yield? Agronomy Journal 110, 715–721. doi:10.2134/agronj2017.09.0540

Msaddak, A., Rejili, M., Durán, D., Rey, L., Imperial, J., Palacios, J.M., Ruiz-Argüeso, T., Mars, M., 2017. Members of Microvirga and Bradyrhizobium genera are native endosymbiotic bacteria nodulating Lupinus luteus in Northern Tunisian soils. FEMS Microbiology Ecology 93, 1–11. doi:10.1093/femsec/fix068

Mukerji, K.G., Manoharachary, C., Singh, J., 2006. Microbial activity in the rhizosphere. Springer Science & Business Media, Berlin.

Mulvaney, R.L., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., 1996. Nitrogen-inorganic forms, in: Methods of Soil Analysis: Part 3 - Chemical Methods. SSSA, Madison, WI, pp. 1123–1184.

Navarrete, A.A., Kuramae, E.E., de Hollander, M., Pijl, A.S., van Veen, J.A., Tsai, S.M., 2013. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. FEMS Microbiology Ecology 83, 607–621. doi:10.1111/1574-6941.12018

Orcutt, B.N., Sylvan, J.B., Knab, N.J., Edwards, K.J., 2011. Microbial Ecology of the Dark Ocean above, at, and below the Seafloor. Microbiology and Molecular Biology Reviews 75, 361–422. doi:10.1128/mmbr.00039-10

Pang, Z., Tayyab, M., Kong, C., Hu, C., Zhu, Z., Wei, X., Yuan, Z., 2019. Liming positively modulates microbial community composition and function of sugarcane fields. Agronomy 9, 1–17. doi:10.3390/agronomy9120808

Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agriculture, Ecosystems and Environment 202, 98–107. doi:10.1016/j.agee.2015.01.005

Pegoraro, R.F., Da Silva, I.R., Novais, R.F., De Sá Mendonça, E., De Oliveira Gebrim, F., Moreira, F.F., 2006. Diffusive flux and bioavailability of micronutrients in soils: Influence of liming, soil texture and green manure. Revista Brasileira de Ciencia Do Solo 30, 859–868. doi:10.1590/S0100-06832006000500012

Pulido-Moncada, M., Lozano, Z., Delgado, M., Dumon, M., Van Ranst, E., Lobo, D., Gabriels, D., Cornelis, W.M., 2018. Using soil organic matter fractions as indicators of soil physical quality. Soil Use and Management 34, 187–196. doi:10.1111/sum.12414

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41, 590–596. doi:10.1093/nar/gks1219

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1993. How a corn plant develops. Special Report n. 48. Iowa State University of Science and Technology. Cooperative Extension Service. Ames, IA, USA.

Rodrigues, J.L.M., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E.D.C., Paula, F.S., Mirza, B., Hamaou, G.S., Tsai, S.M., Feiglf, B., Tiedje, J.M., Bohannan, B.J.M., Nüsslein, K., 2013. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 110, 988–993. doi:10.1073/pnas.1220608110

Shoemaker, H.E., McLean, E.O., Pratt, P.F., 1961. Buffer Methods for Determining Lime Requirement of Soils With Appreciable Amounts of Extractable Aluminum. Soil Science Society of America Journal 25, 274–277. doi:10.2136/sssaj1961.03615995002500040014x

Smith, K.A., Mullins, C.E., 1991. Soil analysis. Physical Methods. Marcel Dekker, New York, p. 7.

Soratto, R.P., Crusciol, C.A.C., 2008. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. Agronomy Journal 100, 261–270. doi:10.2134/agronj2007.0120

Steinberger, Y., Shore, L., 2009. Soil ecology and factors affecting biomass, in: Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations. Springer, pp. 53–61.

Stevenson, F.J., 1994. Humus chemistry: genesis, composition, reactions. John Wiley & Sons.

Strom, N., Hu, W., Chen, S., Bushley, K., 2019. Continuous monoculture shapes root and rhizosphere fungal communities of corn and soybean in soybean cyst nematode-infested soil. Phytobiomes Journal 3, 300–314. doi:10.1094/PBIOMES-05-19-0024-R

Strom, N., Hu, W., Haarith, D., Chen, S., Bushley, K., 2020. Interactions between soil properties, fungal communities, the soybean cyst nematode, and crop yield under continuous corn and soybean monoculture. Applied Soil Ecology 147, 103388. doi:10.1016/j.apsoil.2019.103388

Subbarao, G.V., Yoshihashi, T., Worthington, M., Nakahara, K., Ando, Y., Sahrawat, K.L., Rao, I.M., Lata, J.C., Kishii, M., Braun, H.J., 2015. Suppression of soil nitrification by plants. Plant Science 233, 155–164. doi:10.1016/j.plantsci.2015.01.012

Suleiman, A.K.A., Harkes, P., van den Elsen, S., Holterman, M., Korthals, G.W., Helder, J., Kuramae, E.E., 2019. Organic amendment strengthens interkingdom associations in the soil and rhizosphere of barley (Hordeum vulgare). Science of the Total Environment 695, 133885. doi:10.1016/j.scitotenv.2019.133885

Team, R.C., 2015. R: A Language and environment for statistical computing. R Foundation for Statistical Computing.

Tian, J., Dungait, J.A.J., Lu, X., Yang, Y., Hartley, I.P., Zhang, W., Mo, J., Yu, G., Zhou, J., Kuzyakov, Y., 2019. Long-term nitrogen addition modifies microbial composition and functions for slow carbon cycling and increased sequestration in tropical forest soil. Global Change Biology 25, 3267–3281. doi:10.1111/gcb.14750

Tiritan, C.S., Büll, L.T., Crusciol, C.A.C., Carmeis Filho, A.C.A., Fernandes, D.M., Nascente, A.S., 2016. Tillage system and lime application in a tropical region: Soil chemical fertility and corn yield in succession to degraded pastures. Soil and Tillage Research 155, 437–447. doi:10.1016/j.still.2015.06.012

Tisdall, J.M., Oades, J., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141–163. doi:https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

Unicamp, 2020. Center of Meteorological and Climatic Research Applied to Agriculture. Botucatu: Municipalities climate of São Paulo State [WWW Document]. URL www.cpa.unicamp.br/outras-informacoes/clima_muni_086.html

USDA, 2014. Keys to soil taxonomy. US Gov. Print. Office, Washington, DC, pp. 327–328.

van Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais.

von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant and Soil 171, 1–15. doi:10.1007/BF00009558

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications 10, 1–10. doi:10.1038/s41467-019-12798-y

Waksman, S.A., Gerretsen, F.C., 1931. Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. Ecology 12, 33–60.

Wang, H., Zeng, Y., Guo, C., Bao, Y., Lu, G., Reinfelder, J.R., Dang, Z., 2018. Bacterial, archaeal, and fungal community responses to acid mine drainage-laden pollution in a rice paddy soil ecosystem. Science of the Total Environment 616–617, 107–116. doi:10.1016/j.scitotenv.2017.10.224

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G., 2017. Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China. Applied Soil Ecology 113, 11–21. doi:10.1016/j.apsoil.2017.01.007

Ye, J., Zhang, R., Nielsen, S., Joseph, S.D., Huang, D., Thomas, T., 2016. A combination of biochar-mineral complexes and compost improves soil bacterial processes, soil quality, and plant properties. Frontiers in Microbiology 7, 1–13. doi:10.3389/fmicb.2016.00372

Yoder, R.E., 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses 1. Agronomy Journal 28, 337–351. doi:https://doi.org/10.2134/agronj1936.00021962002800050001x

Yun, Y., Wang, H., Man, B., Xiang, X., Zhou, J., Qiu, X., Duan, Y., Engel, A.S., 2016. The relationship between pH and bacterial communities in a single karst ecosystem and its implication for soil acidification. Frontiers in Microbiology 7, 23–32. doi:10.3389/fmicb.2016.01955

Zhu, L., Wei, Z., Yang, T., Zhao, X., Dang, Q., Chen, X., Wu, J., Zhao, Y., 2020. Core microorganisms promote the transformation of DOM fractions with different molecular weights to improve the stability during composting. Bioresource Technology 299, 122575. doi:10.1016/j.biortech.2019.122575

Zoca, S.M., Penn, C., 2017. An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. Advances in Agronomy 144, 1–44. doi:10.1016/bs.agron.2017.03.001

SUPPLEMENTARY MATERIAL

Table S1. Soil physicochemical properties of the field experimental prior to experiment installation.

Soil properties		Depth	Unit	Value
Sand		0.00−0.20 m		545
		0.20−0.40 m		513
Clay		0.00−0.20 m	g kg^{-1}	347
		0.20−0.40 m		360
Silt		0.00−0.20 m		108
		0.20−0.40 m		127
Porosity	Macro	0.00−0.20 m		4.80
		0.20−0.40 m		4.60
	Micro	0.00−0.20 m	m^3 m^{-3}	18.9
		0.20−0.40 m		18.3
	Total	0.00−0.20 m		23.7
		0.20−0.40 m		22.9
Bulk density			Mg m^{-3}	1.13
pH ($CaCl_2$)			–	4.20
SOM†			g kg^{-1}	21.0
$P_{available\ (resin)}$			mg kg^{-1}	9.20
Exchange	Ca^{2+} (resin)		$mmol_c$ kg^{-1}	14.0
	Mg^{2+} (resin)			5.00
	K^{+} (resin)			1.20
	Al^{3+} (KCl)			5.60
H+Al		0.00−0.20 m		36.8
ECEC‡				58.0
BS§			%	37.0
S-SO_4^{2-} ($Ca(H_2PO_4)_2$)				3.65
Fe (DTPA-TEA)			mg kg^{-1}	32.2
Cu (DTPA-TEA)				5.00
Mn (DTPA-TEA)				21.8
Zn (DTPA-TEA)				1.40

†SOM, soil organic matter; ‡ECEC, effective cation exchange capacity; §BS, base saturation;

Table S2. Crop history and the treatment application scheme throughout the experimental period (2002–2019).

Crop season	Sowing	Crops	Fertilizer (kg ha^{-1})[†]						Treatment application[‡]
			BF					TF	
			N	P	K	S	Zn	N	
2002/2003	Nov.	*Oriza sativa*	24	37	40	14	1.5	50	L: 2.7 Mg ha^{-1} (71% ECCE) PG: 2.1 Mg ha^{-1}
	Apr.	*Avena strigosa*	20	17	17	9	–	–	
2003/2004	Jan.	*Phaseolus vulgaris*	24	37	40	14	1.5	110	L: 2.0 Mg ha^{-1} (71% ECCE) PG: 2.1 Mg ha^{-1}
	Apr.	*Avena strigosa*	8	17	17	14	–	–	
2004/2005	Nov.	*Arachis hypogaea*	24	37	40	–	1.5	–	–
	Apr.	*Avena sativa*	8	17	17	14	–	–	
2005/2006	Nov.	*Arachis hypogaea*	24	37	40	–	1.5	–	–
	May	*Avena sativa*	8	17	17	14	–	–	
2006/2007	Feb.	*Zea mays* + *Urochloa brizantha*	24	37	40	–	–	100	–
	-	*Urochloa brizantha*	–	–	–	–	–	–	
2007/2008	Dec.	*Zea mays* + *Urochloa brizantha*	24	37	40	–	–	100	–
	-	*Urochloa brizantha*	–	–	–	–	–	–	
2008/2009	Dec.	*Glycine max*	10	22	42	–	–	–	–
	Jun.	*Avena strigosa*	-			–	–	–	
2009/2010	Oct.	*Glycine max*	10	22	42	–	–	–	–
	Mar.	*Sorghum vulgare*	-			–	–	–	
2010/2011	Nov.	*Zea mays*	30	44	50	–	–	150	L: 2.0 Mg ha^{-1} (88% ECCE) PG: 2.1 Mg ha^{-1}
	Apr.	*Crambe abyssinica*	10	17	21	–	–	–	
	Sept.	*Vigna unguiculata*	20	26	25	–	–	50	
2011/2012	Nov.	*Zea mays*	30	44	50	–	–	150	–
	May	*Crambe abyssinica*	10	17	21	–	–	–	
	Sept.	*Vigna unguiculata*	30	44	50	–	–	50	
2012/2013	Jan.	*Pennisetum glaucum*	–	–	–	–	–	–	–
	Apr.	*Triticum aestivum*	75	31	33	11	1.2	–	
2013/2014	Nov.	*Phaseolus vulgaris*	10	22	42	11	1.2	100	–
	Mar.	*Triticum aestivum*	75	31	33	11	1.2	–	
2014/2015	Dec.	*Phaseolus vulgaris*	10	22	42	11	1.2	100	–
	Mar.	*Urochloa brizantha*	–	–	–	–	–	–	
2015/2016	-	*Urochloa brizantha*	–	–	–	–	–	–	–
	-	*Urochloa brizantha*	–	–	–	–	–	–	
2016/2017	Oct.	*Glycine max*	10	22	42	–	–	–	L: 13 Mg ha^{-1} (69% ECCE) PG: 10 Mg ha^{-1}
	Mar.	*Zea mays* + *Urochloa ruziziensis*	24	37	40	11	–	100	
2017/2018	Oct.	*Glycine max*	10	22	42	–	–	–	–
	Mar.	*Zea mays* + *Urochloa ruziziensis*	24	37	40	11	–	100	
2018/2019	Oct.	*Glycine max*	10	22	42	–	–	–	–
	Mar.	*Zea mays* + *Urochloa ruziziensis*	24	37	40	11	–	100	

[†]BF: base fertilization and TF: topdressing fertilization; [‡]L: lime, PG: phosphogypsum and ECCE: effective calcium-carbonate equivalent.

Below the dotted line corresponds to the area's history after the last soil amendments reapplication (2016).

SUPPLEMENTARY MATERIAL AND METHODS

- *Lime and Phosphogypsum rates calculations*

The dolomitic lime rate (DLR) was calculated to increase the base saturation (BS) in the 0.00–0.40 m layer to 70%, by using Eq. (1), adapted from van Raij et al. (1997), and then, multiplied by a factor to obtain the double of the recommended dose:

$$\mathrm{DLR\ (Mg\ ha^{-1})} = \frac{(\mathrm{BS_2 - BS_1}) \times \mathrm{CEC})_{0.0-0.40\ m}}{\mathrm{ECCE} \times 10} \times 2 \quad (1)$$

where BS_2 is the estimated base saturation (70%); and BS_1 is the base saturation determined by soil chemical analysis for each layer and calculated according to Eq. (2):

$$\mathrm{BS_1\ (\%)} = \frac{\left(\mathrm{Ca^{2+} + Mg^{2+} + K^{+}}\right) \times 100}{\mathrm{CEC}} \quad (2)$$

where Ca^{2+}, Mg^{2+} and K^{+}, are the contents of soil exchangeable bases, and CEC is the cation exchange capacity, which was calculated by Eq. (3):

$$\mathrm{CEC\ (mmol_c kg^{-1})} = \left(\mathrm{Ca^{2+} + Mg^{2+} + K^{+} + total\ acidity\ pH\ 7\ (H + Al)}\right) \quad (3)$$

The first three PG rates (PGR) applied (2002, 2004 and 2010) were calculated according to the methodology proposed by (van Raij et al., 1997) showed in Eq. (4):

$$\mathrm{PGR\ (Mg^{-1})} = \frac{(6 \times \mathrm{clay\ content})}{1000} \quad (4)$$

where clay content (g kg^{-1}) corresponds to depth of 0.20–0.40 m. The last PG reapplication (2016) was calculated by Eq. (5) according to the new methodology proposed by (Caires and Guimarães, 2018), in order to raise Ca^{2+} saturation to 60% in the CEC at the 0.20–0.40 m soil layer:

$$\mathrm{PGR\ (Mg^{-1})} = \left((0.6 \times \mathrm{CEC}) - Ca^{2+}\right) \times 0.64 \quad (5)$$

where CEC and exchangeable Ca^{2+} corresponds to the depth 0.20–0.40 m.

- *Aboveground dry matter production*

Aboveground dry matter production of maize was determined after harvest. In 15m^2 area, plants were collected and separated of grains. The remaining straw was dried and subsequently weighted. Ruzigrass continued to vegetate after the corn harvest and its aboveground was determined two months after maize Harvest,

collecting 2 m^2 per plot, and subsequently dried and weighted. Both dry matter production was converted to Mg ha^{-1}.

REFERENCES

Caires, E.F., Guimarães, A.M., 2018. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. Agronomy Journal 110, 1987–1995. doi:10.2134/agronj2017.11.0642

van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendações da adubação e calagem para o Estado de São Paulo. Instituto Agronômico/Fundação IAC Campinas.

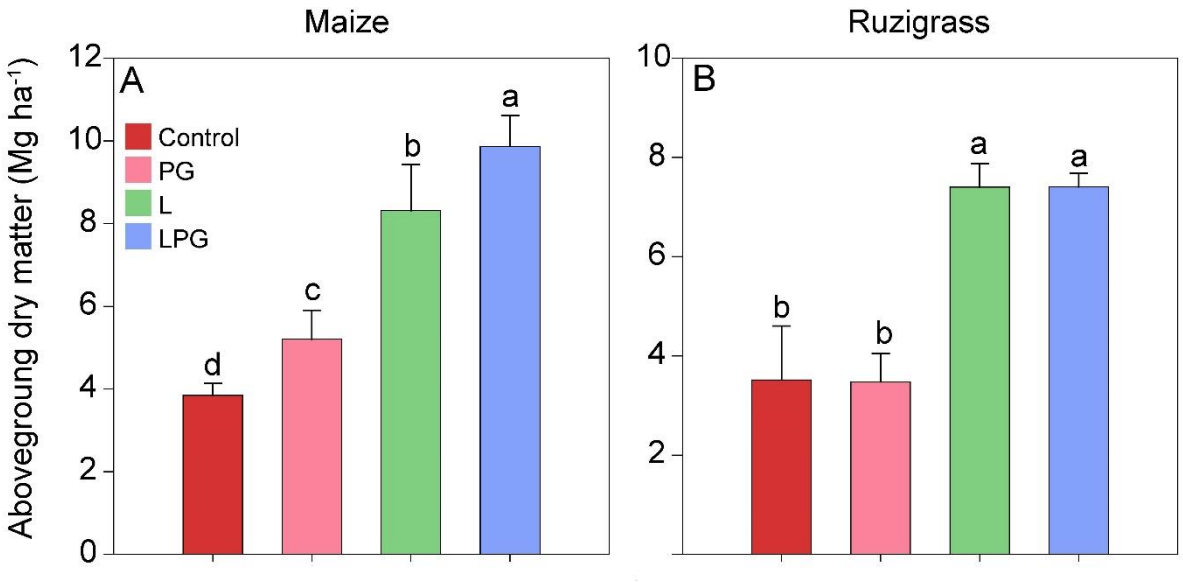


Figure S1. Aboveground dry matter production of maize (A) and ruzigrass (B) cultivated in a long-term soil with amendments (lime; PG, phosphogypsum; LPG, lime + phosphogypsum) applied in soil surface.

Table S3. Statistical parameters by ANOVA and Student's t-test at $p \leq 0.05$ significance level according to the soil amendments [control (no soil amendment application), lime (L), phosphogypsum (PG), and lime + phosphogypsum (LPG)] for soil organic matter fractions, and soil physicochemical parameters.

Statistical parameters	POC	MOC	TOC	C-HA	C-FA	C-HU	pH ($CaCl_2$)	Al^{3+}	NO_3^-	NH_4^+	P (resin)	K^+
Soil amendments	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.13	1.43	1.47	0.09	0.03	0.85	0.27	1.14	1.71	2.29	6.78	0.72
CV (%)	7.58	7.65	7.21	0.09	9.48	5.46	3.32	15.0	3.89	6.85	10.6	12.8

	Ca^{2+}	Mg^{2+}	S-SO_4^{2-}	Fe	Mn	Cu	Zn	MWD	SBD	mP	MP
Soil amendments	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.261	<0.001
LSD	2.96	1.87	3.67	1.60	2.71	0.07	0.30	0.19	0.09	3.95	2.02
CV (%)	3.77	6.60	10.6	3.6	4.77	1.97	5.59	5.01	3.54	7.93	12.0

Statistical parameters by ANOVA and LSD test at $p \leq 0.05$.

Particulate organic carbon (POC), mineral- associated organic carbon (MOC), total organic carbon (TOC), organic C in humic acid (C-HA), organic C in fulvic acid (C-FA), Organic C in humin (C-HU), exchangeable Al^{3+}, nitrate (NO^{3-}), ammonium (NH_4^+), phosphorus available (P_{resin}), exchangeable potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), sulfur-sulfate (S-SO_4^{2-}) and available iron (Fe), manganese (Mn), cupper (Cu), zinc (Zn), mean weight diameter (MWD), soil bulk density (SBD), microporosity (mP) and macroporosity (MP).

Table S4. Summary of affected taxa by treatments.

Taxa		Number of taxa† with significant shifts on the relative abundance according to the treatments				Distinct taxa per phylum	
		Control	PG	L	LPG	Genus	Species
Kingdom	Bacteria	31	21	19	14	55	-
Phylum	Acidobacteriota	4	3	3	1	6	-
	Actinobacteriota	10	4	6	4	15	-
	Armatimodota	1				1	-
	Chloroflexi	4	3	2	1	5	-
	Firmicutes	1	3			3	-
	Gemmatimodota	1	1			1	-
	Planctomycetota	3	2	1	1	4	-
	Proteobacteria	7	4	7	7	19	-
	Verrucomicrobiota		1			1	-
Kingdom	Fungi	28	24	24	32	57	75
Phylum	Ascomycota	21	22	22	26	46	63
	Basidiomycota	6	1	1	4	8	9
	Chytridiomycota	1				1	1
	Mortierellomycota		1	1	2	2	2

†Genus level for bacteria, and species level for fungi; same taxa can occur simultaneously between treatments

Table S5. List of bacteria (genus level) significantly affected by treatments.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Bacteria	Acidobacteriota	Blastocatellia	Pyrinomodales	Pyrinomodaceae	RB41	un
Bacteria	Acidobacteriota	Acidobacteriae	Bryobacterales	Bryobacteraceae	Bryobacter	un
Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup_1)	Terracidiphilus	un
Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup_1)	Acidipila	un
Bacteria	Acidobacteriota	Vicinibacteria	Vicinibacterales	Vicinibacteraceae	Luteitalea	un
Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Koribacteraceae	Candidatus_Koribacter	un
Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Conexibacter	un
Bacteria	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Luedemannella	un
Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	un
Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Frankiaceae	Jatrophihabitans	un
Bacteria	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Crossiella	un
Bacteria	Actinobacteriota	Actinobacteria	Kineosporiales	Kineosporiaceae	Quadrisphaera	un
Bacteria	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Kitasatospora	un
Bacteria	Actinobacteriota	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	un
Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Sinomos	un
Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces	un
Bacteria	Actinobacteriota	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	un
Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Intrasporangiaceae	Janibacter	un
Bacteria	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptacidiphilus	un
Bacteria	Actinobacteriota	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Streptosporangium	un
Bacteria	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	Catellatospora	un
Bacteria	Armatimodota	Chthonomodetes	Chthonomodales	Chthonomodaceae	Chthonomos	un
Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	G12-WMSP1	un
Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	1921-2	un
Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	HSB_OF53-F07	un
Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	1921-3	un
Bacteria	Chloroflexi	Aerolineae	SBR1031	A4b	OLB13	un
Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Paenisporosarci	un
Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomodales	Sporomusaceae	Aerosinus	un
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1	un
Bacteria	Gemmatimodota	Gemmatimodetes	Gemmatimodales	Gemmatimodaceae	Roseisolibacter	un
Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae	Aquisphaera	un
Bacteria	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	Gemmata	un
Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae	Tundrisphaera	un
Bacteria	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	Pirellula	un

Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Nitrobacter	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	un
Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	Povalibacter	un
Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	Steroidobacter	un
Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidicaldus	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia-Caballeronia-Paraburkholderia	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamodaceae	Ramlibacter	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium	un
Bacteria	Proteobacteria	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomodaceae	Ellin6067	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamodaceae	Azohydromos	un
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomodales	Rhodanobacteraceae	Rhodanobacter	un
Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomodaceae	MND1	un
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomodales	Rhodanobacteraceae	Dokdonella	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium-Methylorubrum	un
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Labraceae	Labrys	un
Bacteria	Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	ADurb.Bin063-1	un

Unclassified (un)

Table S6. List of fungi (species level) significantly affected by treatments.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	acutatum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae sedis	Acremonium	persicinum
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Humicola	olivacea
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Paracremonium	inflatum
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	raperi
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Xenomyrothecium	tongaense
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	pseudensiforme
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Xepicula	leucotricha
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	Metarhizium	marquandii
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae sedis	Acremonium	curvulum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Gibberella	intricans
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	Clonostachys	rosea
Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Chloridium	aseptatum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae sedis	Acremonium	furcatum
Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Scytalidium	circitum
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariales_fam_Incertae_sedis	Staphylotrichum	coccosporum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Sarcopodium	circinosetiferum
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	terricola
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraconiothyrium	cyclothyrioides
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporales_fam_Incertae sedis	Pseudorobillarda	phragmitis
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	rifaii
Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Immersiella	caudata
Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	verticillata
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	compactum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	Valsonectria	pulchella
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Phaeosphaeria	caricis
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	iizukae
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	Claviceps	chloridicola
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Thyridariaceae	Roussoella	solani
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	hetheringtonii
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Volutella	consors
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertae sedis	Trichothecium	crotocinigenum
Fungi	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	Colletotrichum	aciculare
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	turrialbense
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Penicillifer	diparietisporus
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Arcopilus	cupreus
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Pseudopithomyces	rosae
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	fuscoviridis
Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Knufia	mediterranea

Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Acrophialophora	levis
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	aculeatus
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	wyomingensis
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Albifimbria	verrucaria
Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Dinemasporium	americanum
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Stachybotrys	limonispora
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	sinuosum
Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Dinemasporium	parastrigosum
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraconiothyrium	brasiliense
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Cordyceps	bassia
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	wentii
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	bilaiae
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Escovopsis	kreiselii
Fungi	Ascomycota	Eurotiomycetes	Onygeles	Onygeles_fam_Incertae_sedis	Chrysosporium	fluviale
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	Flabellascoma	cycadicola
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	cuneirostrum
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	caninus
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae	Pyrenochaetopsis	leptospora
Fungi	Ascomycota	Laboulbeniomycetes	Pyxidiophorales	Pyxidiophoraceae	Pyxidiophora	arvernensis
Fungi	Ascomycota	Eurotiomycetes	Onygeles	Onygeceae	Arachnotheca	glomerata
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	chlamydosporus
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_fam_Incertaesedis	Acremonium	roseolum
Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	Arthrobotrys	dactyloides
Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	Dactylella	arudii
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	Saitozyma	podzolica
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Hanella	oryzae
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Hanella	zeae
Fungi	Basidiomycota	Tremellomycetes	Trichosporoles	Trichosporoceae	Apiotrichum	loubieri
Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Trechispora	bispora
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Hymenogastraceae	Hebeloma	crustuliniforme
Fungi	Basidiomycota	Tremellomycetes	Trichosporoles	Trichosporoceae	Trichosporon	insectorum
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	Physalacria	maipoensis
Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma	terrea
Fungi	Chytridiomycota	Rhizophlyctidomycetes	Rhizophlyctidales	Rhizophlyctidaceae	Rhizophlyctis	rosea
Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	alpi
Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	ambigua

CHAPTER 4

LONG-TERM LIME AND GYPSUM AMENDMENT INCREASE NITROGEN FIXATION AND DECREASE NITRIFICATION AND DENITRIFICATION GENE ABUNDANCES IN THE RHIZOSPHERE AND SOIL IN A TROPICAL NO-TILL INTERCROPPING SYSTEM[4]

Published in: Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375, 114476. doi:10.1016/j.geoderma.2020.114476

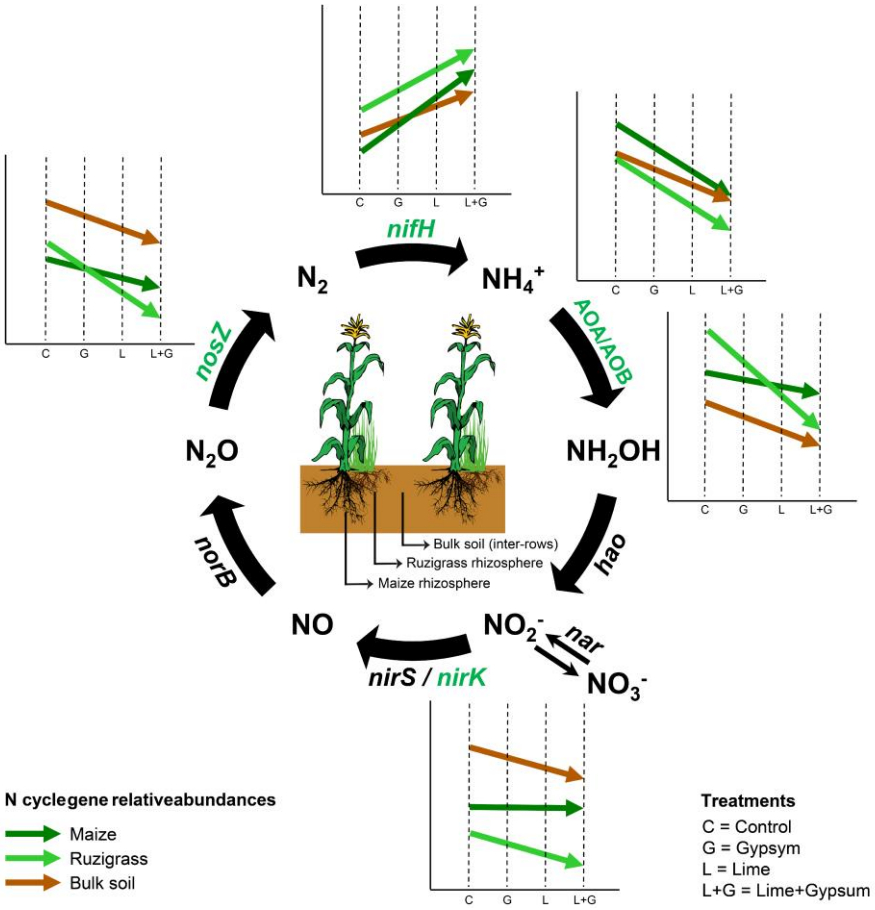
Abstract

Liming is widely used to decrease soil acidity, and the application of lime alone or in combination with other amendments, such as gypsum, is a viable agricultural practice to improve soil nutrient status and crop yield. However, the effects of applying lime and gypsum alone or in combination on the microbial population and N cycle in intercropped no-till tropical systems are largely unknown. Here, we determined the lasting effects of applying lime and gypsum individually or in combination on soil chemical properties, N uptake by intercropped plants, maize yield, archaeal and bacterial abundances, and N cycle genes in the maize and ruzigrass rhizospheres in a long-term field experiment in tropical soil with a no-till maize and forage ruzigrass intercropping system. Our results showed that the application of lime or lime + gypsum increased soil fertility and the gene abundances of microorganisms responsible for biological nitrogen fixation and reduced gene abundances of nitrification and denitrification in the soil and rhizosphere of ruzigrass and maize. The accompanying increases in Ca^{2+} and Mg^{2+} availability, reduced Al^{3+} levels, and balance of micronutrient availability, mainly Mn, in the soil strongly influenced the responses of N cycle genes and enhanced plant N-acquisition and maize yield.

Keywords: Soil quality, crops, *Zea mays*, *Urochloa ruziziensis*, N cycle genes

[4] Chapter written in accordance with the rules of Geoderma.

Graphical abstract



4.1 INTRODUCTION

Continuous crop cultivation under tropical conditions typically leads to severe declines in soil quality and, consequently, lower crop productivity (Holland et al., 2018). Soil acidity is one of the primary factors limiting crop development and productive potential (FAO, 2015). Globally, approximately 50% of agricultural areas are affected by problems related to soil acidity (Von Uexküll and Mutert, 1995). Most of these soils are in tropical and subtropical regions and, due to the high degree of weathering, also feature low cation exchange capacity (CEC) and low base saturation (BS) (Allen et al. 2007). In addition, these tropical acidic soils contain high levels of elements that are toxic to plants, such as exchangeable aluminum (Al^{3+}) and manganese (Mn) (Caires et al., 2011; Crusciol et al., 2016; 2019).

There is global interest in more sustainable soil management systems, including the reevaluation of existing management practices (Gibbons et al., 2014; Holland et

al., 2018). Liming is an extremely important agricultural practice, particularly in tropical soils, that neutralizes soil acidity, increases nutrient availability, supplies calcium (Ca^{2+}) and magnesium (Mg^{2+}) and relieves the toxicity of some elements, especially in the topsoil layers (Caires et al. 2011). To increase the efficiency of liming in tropical soils, agricultural gypsum can be added (Inagaki et al., 2016; Carmeis Filho et al., 2017; Crusciol et al., 2016). Gypsum has been widely used in the recovery of sodic soils or as a source for Ca and S (Zoca and Penn, 2017), but more recently, it has been widely applied on slightly dispersed and highly acid soils. Unlike liming, gypsum amendment does not correct soil acidity but provides Ca^{2+} and sulfur (S) and reduces Al^{3+} availability throughout the soil profile (Soratto et al., 2010; Caires et al., 2011; Carmeis Filho et al., 2017). The improvement in the soil profile by gypsum benefits crop root system development (Rosolem et al., 2017) and changes the nutrient dynamics and use due to the increasing nutrient cycling and nutrient uptake in agricultural systems (Holland et al., 2018).

The increase in soil pH induced by liming, which directly impacts soil transformation processes, has been studied extensively (Zhang et al., 2017; Liu et al., 2018; Guo et al., 2019; Pang et al., 2019). The changes induced by liming directly influence the nitrogen (N) supply to plants and N loss to the atmosphere via N_2O emissions and NH_3 volatilization or to groundwater by NO_3^- leaching (Liu et al., 2010; Zhang et al., 2017). According to Jarvis (1984) liming also promotes crop growth due to improved nodulation and increased N fixation through the increased abundance of compatible rhizobia and reduced constraints inhibiting the infection and nodulation of the host plant. It has been shown that liming can either improve N mineralization and subsequent beneficial effects on for cropping systems, including minimizing the inputs of nitrogen fertilizers (Holland et al., 2018). It is known that the soil microbiota influences soil element solubility and availability (Stiehl-Braun et al., 2011; Wachendorf, 2015). In addition, nutrient availability affects microbial metabolic activity (Navarrete et al., 2013). However, the exact mechanisms by which these parameters influence the associated microbial communities remain unclear.

Studies of the long-term effects of lime and gypsum amendment on the composition of the microbial soil community, particularly those related to the abundances of N cycle, are extremely scarce. This information, particularly the long-term effects of lime and gypsum application in agricultural soils, is essential for the development of sustainable management practices that avoid N losses to the

environment (Kemmitt et al., 2006; Liu et al., 2018). Moreover, comprehensive investigations of the long-term effects of liming and other soil amendment practices could bring important information on how to reduce the excessive use of fertilizers, due to improved soil quality and increase the sustainability of current nutrient management practices. According to Holland et al., (2018), the impact of liming results in increased activity of soil biota. Consequently, the rate of nutrient cycling processes is increased, and soil function is impacted. The interaction of the changes in soil chemistry promoted by lime and gypsum with the soil microbiota is an important open question, especially in intensive farming systems with a diversity of plants growing at the same time (intercropping). No-till intercropping systems are characterized by alternation between cash crops and forage production during the growing season with or without animal grazing in the off-season (Franzluebbers et al., 2019). These systems can increase soil quality by improving soil chemical, physical and biological properties (Pariz et al., 2017), biodiversity conservation (Kim et al., 2020), and nutrient cycling by plants and soil organic matter (Franzluebbers et al., 2019) and reduce greenhouse gas emissions (Savian et al., 2014).

Nitrogen can be the most limiting nutrient on the crop development in tropical regions. Successive grass-only cultivation can compromise the sustainability of agroecosystems due to soil N depletion through nutrient removal (Garcia et al., 2016). The amount of N required by crops varies according to the environmental conditions and characteristics of the plants used in rotation; however, in crop systems that include only grasses, such as intercropping maize and tropical grass, high amount of N is required (Mateus et al., 2020). These characteristics are extremely important for agriculture in tropical regions around the world. The sustainability of these soils depends on the correct soil acidity management and the input of organic matter content provided by crops in rotation system.

Here, we investigated the long-term effects of lime and gypsum application on soil fertility and their influences on N cycle genes in the soil and rhizosphere of cover and cash crops in a tropical soil. To better understand these impacts in intercropped systems, we analyzed the rhizospheres of each plant and bulk soil separately. We hypothesized that the combined application of lime and gypsum would affect the abundances of N cycle genes in the agricultural system due to changes in soil chemical properties. To test this hypothesis, the following questions were addressed: (*i*) What are the main changes in properties in soil amended with lime, gypsum and lime +

gypsum? (*ii*) What are the impacts of these amendments on total archaea and bacteria and on the relative abundance of N cycle genes in the rhizosphere of each plant species and the soil? (*iii*) What are the main soil chemical properties that influence N cycle genes? (*iv*) How do changes in soil fertility and the N cycle affect mineral nitrogen availability for plant uptake and maize yield?

4.2 MATERIAL AND METHODS

4.2.1 Field description and sample collection

A long-term lime and gypsum application field experiment was initiated in October 2002 at the Experimental Farm Station in Botucatu, southeast of São Paulo State, Brazil (22° 83' 3" S, 48° 42' 64" W, elevation 765 m above sea level). The soil was classified as a sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2014). According to Köeppen's classification, the climate is Cwa type, which corresponds to a tropical altitude with a dry winter and a hot, wet summer (Alvares et al., 2013). The long-term (1956-2019) average annual temperatures are 26.1 °C maximum and 15.3 °C minimum, with a 20.7 °C average. The annual rainfall average is approximately 1,360 mm (Unicamp, 2020). Climatological data during the experiment period were presented in Supplementary Fig. S1.

Prior to establishing the experiment in 2002, the soil chemical properties and mineral soil fraction were determined (0.00–0.20 m depth), according to van Raij et al. (2001) and Kiehl (1979), respectively. The following results were obtained: soil organic matter (SOM): 21 g kg^{-1}; pH ($CaCl_2$): 4.2; phosphorus (P_{resin}): 9.2 mg kg^{-1}; exchangeable potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}): 1.2, 14, and 5 $mmol_c$ kg^{-1}, respectively; total acidity in pH7 (H+Al): 37 $mmol_c$ kg^{-1}, CEC: 57 $mmol_c$ kg^{-1}; BS: 35%; sand, silt and clay contents: 540, 110, and 350 g kg^{-1}, respectively. In the subsoil (0.20–0.40 m), the clay content was 360 g kg^{-1}. The bulk density at depth 0.00–0.20 m was 1.128 t m^{-3}.

4.2.2 Experimental design and treatments

The experiment design was a complete randomized block with four replications. The plots had four treatments: (*i*) a control (natural soil conditions; 17 years without soil amendment and intensive crop cultivation with fertilizer inputs); (*ii*) gypsum (10.0

Mg ha^{-1}); (*iii*) lime (13.04 Mg ha^{-1}); (*iv*) lime (13.04 Mg ha^{-1}) + gypsum (10.0 Mg ha^{-1}) application. A graphical scheme of the crop system and respective treatments and samplings are shown in Fig. 1.

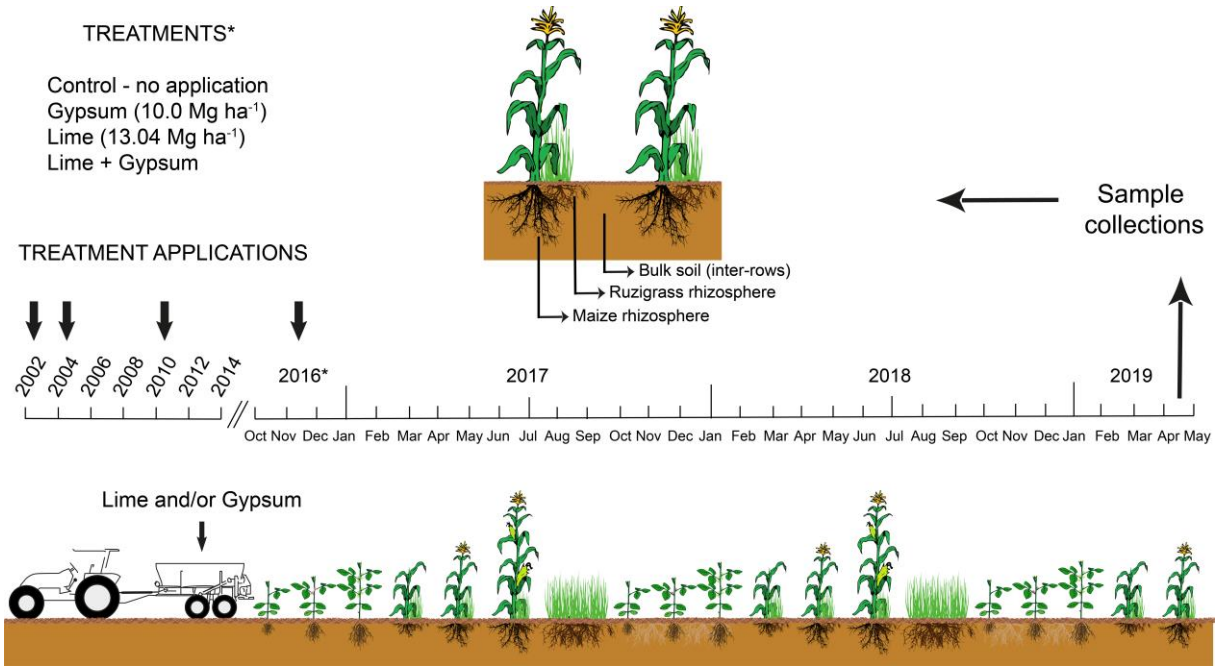


Figure 1. Schematic graphic representing the timeline of the field experiment and the respective treatments and sample collections.

The dolomitic lime dose (DLD) was calculated to increase the base saturation (BS) in the topsoil (0.00–0.20 m) to 70%, according to the method of Quaggio and van Raij (1997), and then multiplied by a factor 2 (to obtain de double of the recommeded dose) as shown in Eq. (1):

$$DLD\ (Mg\ ha^{-1}) = (CEC \times (BS_2 - BS_1) / (10 \times ECCE)) \times 2 \quad (1)$$

where BS_2 is the estimated base saturation (70%), and BS_1 is the base saturation measured in the soil analysis as shown in Eq. (2). ECCE is the effective calcium carbonate equivalents.

$$BS_1\ (\%) = (Ca^{2+} + Mg^{2+} + K^{+}) \times 100/CEC \quad (2)$$

where Ca^{2+}, Mg^{2+}, and K^{+} are basic exchangeable cations ($mmol_c\ kg^{-1}$), and CEC is the total cation exchange capacity, calculated as shown in Eq. (3):

$$CEC\ (mmol_c\ kg^{-1}) = Ca^{2+} + Mg^{2+} + K^{+} + (H+Al) \quad (3)$$

The gypsum dose applied in 2002, 2004 and 2010 were calculated by Eq. (4) (Quaggio and van Raij, 1997):

$$GD\ (Mg\ ha^{-1}) = 6 \times clay\ content\ (g\ kg^{-1}) / 1000 \quad (4)$$

where clay content corresponds to the soil layer of 0.20 - 0.40 m. In 2016, the gypsum dose was calculated based on the new methodology proposed by Caires and Guimarães (2018), with Eq. (5):

$$GD\ (Mg\ ha^{-1}) = [(0.6 \times CEC) - Ca^{2+})] \times 0.64 \quad (5)$$

where CEC and Ca^{2+} were calculated based on 0.20 - 0.40 m soil layer.

4.2.3 Lime and gypsum application, tillage and crop management

During the study period, the treatments were applied four times (2002, 2004, 2010 and 2016) (Fig. 1). The criterion for reapplying treatments is based on annual assessments of soil fertility when base saturation of exclusive lime treatment reaches value ≤ 50%. The present study corresponds to third year after the last reapplication (2016), characterized by being a long-term residual effect. Different crops were cultivated during the growing season and the off season from 2002 to 2019. Details of the previous crop sequences and lime and gypsum applications as well as the characteristics of these amendments are shown in Supplementary Table S1. Since the experiment was implemented, it was cultivated under no-tillage system (NTS) and all lime and gypsum applications were performed superficially, except for the first application in 2002, when no-tillage began.

In March 2019 (off season), maize (*Zea mays* L.) was sowing intercropped with ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (Syn. *Brachiaria ruziziensis* Germ. & Evrard)]. Fertilization was performed at sowing time with rates of 350 kg ha^{-1} of $N–P_2O_5–K_2O$ 08–28–16 and 100 kg N ha^{-1} as ammonium sulfate in topdressing (Cantarella et al., 1997) when maize was at V_4 phenological stage (Ritchie et al., 1993). The field plots consisted of 14 rows that were 9 m long and spaced 0.45 m apart, a total plot area of 56.7 m^2. Plots were separated by a space of 8 m to avoid contamination during treatments applications and subsequent cropping seasons. Eight soil subsamples per plot were randomly obtained from rhizosphere of maize and ruzigrass and bulk soil (between rows)to form a composite sample . Samples were collected when maize was at V_{10} phenological stage (Ritchie et al., 1993). The rhizosphere samples were strictly defined as the soil within 2 mm of the root surface (DeAngelis et al., 2009). After gently shaking the roots to remove loosely attached soil clumps, the rhizosphere samples were carefully collected by brushing the remaining

soil from the roots (Schlemper et al., 2017). The bulk soil was collected between maize + ruzigrass rows at the depth 0.00–0.10 m.

4.2.4 Soil chemical properties analysis

Eight soil subsamples were randomly obtained at depth 0.00-0.10 m from useable areas of each plot to form a composite sample and were processed according to the standard methods for Brazilian tropical soils (van Raij et al., 2001). Samples were air-dried and homogenized using a 2 mm sieve. The soil organic carbon (SOC) was determined by the colorimetric method using a sodium dichromate solution according the method proposed by Heanes (1984). The pH values were measured in 0.01 M $CaCl_2$ (the most stable pH used in soil analysis). The exchangeable cations (K^+, Ca^{2+}, and Mg^{2+}) and available P-phosphate were extracted using ion exchange resins and, in the extract, P as orthophosphate was determined colorimetrically, and cations by atomic absorption spectrometry (Shimadzu AA-7000) (van Raij et al. 2001). Soil sulfur-sulfate ($S\text{-}SO_4^{2-}$) extraction were performed by calcium phosphate (0.01 mol L^{-1}) in a 1:2.5 soil/solution ratio and later determined by the turbidimetric method using $BaSO_4$ (Bardsley and Lancaster, 1960). The cationic micronutrients [iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn)] were extracted by solution (pH 7.3) containing 0.005 mol L^{-1} of diethylenetriaminepentaacetic acid (DTPA), 0.1 mol L^{-1} of triethanolamine (TEA) and 0.01 mol L^{-1} of $CaCl_2$ and determined by atomic absorption spectrometry. Potential acidity (H+Al) was estimated by the Shoemaker-McLean-Pratt buffer solution method (Shoemaker et al., 1961). The exchangeable Al^{3+} was extracted with 1 mol L^{-1} KCl (pH 7.0) at 1:10 soil/solution ratio, kept under agitation for 15 min and determined by titration with a 0.025 mol L^{-1} NaOH solution. After extraction, the content of ammonium (NH_4^+) and nitrate (NO_3^-) was determined by colorimetry by sodium salicylate method and vanadium chloride reduction, respectively (Mulvaney, 1996; Miranda et al., 2001). Total inorganic nitrogen (N_i) was obtained by summing the measured concentrations of mineral forms (NH_4^+ and NO_3^-).

4.2.5 Soil DNA extraction

Total DNA extraction from 250 mg of soil sample was performed for each sampled point (maize and ruzigrass rhizospheres and bulk soil) using the PowerSoil

DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), according to manufacturer's protocol. DNA quality and concentration were measured using NanoDrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, USA) and 1% sodium boric acid agarose gel electrophoresis (Brody and Kern, 2004).

4.2.6 Quantitative PCR analyses

The abundance of total archaea and bacteria, nitrogen fixers, nitrifiers and denitrifiers were estimated by quantitative PCR targeting archaeal and bacterial genes (16S rRNA), nitrogenase gene (*nifH*), ammonia monooxygenase gene (*amoA*) of archaea (AOA) and bacteria (AOB), nitrite reductase gene (*nirK*) and nitrous oxide reductase gene (*nosZ*), respectively. Analyses were performed using the StepOnePlus™ Real-Time PCR System with 96-well plates (Applied Biosystems, Foster City, CA, USA). Standard curves were created after serial dilutions of known gene quantity, amplified by PCR previously. Strains that were used to construct the standard curves, primers, and reaction conditions for genes amplifications are described in Supplementary Table S2. Analyses were performed in triplicate, and for each reaction was used 5μL absolute qPCR SYBR Green/ROX qPCR Master Mix (2×) (Abgene, Epsom, UK); 1 μL of each primer (30 μM); 1.5 μL of sterilized water, 1 μL template DNA and 0.5 μL bovine serum albumin (BSA; 10 mg ml^{-1}). Analysis of melting curves were performed from 68 to 95°C, and all standard curves will have R^2 greater than 0.98 as well efficiency between 98-102%. Results were analyzed using the StepOnePlus™ Real-Time software (Applied Biosystems, Foster City, CA, USA). Abundance data from each sample were transformed into the number of copies of DNA g $soil^{-1}$.

4.2.7 Determination of plant nitrogen content and crop yield

At the maize flowering, phenological stage R_1 (Ritchie et al., 1993), the leaf at the ear base (only the central third parts) were sampled from 30 random maize plants within the useful area of the plot (Cantarella et al. 1997), totaling 30 leaves per plot for N determination (Malavolta et al. 1997). The same was done for ruzigrass plants. The maize grain yield (moisture content of 130 g kg^{-1} of water) was evaluated at harvest maize stage.

4.2.8 Statistical analysis

The results were subjected to the outliers' test followed by Anderson-Darling normality (Nelson et al., 1998), and to evaluate the homogeneity the Levene's test was applied by the Minitab statistical program. Then, the means were subjected to the analysis of individual variance ANOVA by the F test ($p \leq 0.05$), and when there was significant difference, the means were compared by the LSD test ($p \leq 0.05$). Redundancy analysis (RDA) was used to determine the correlation between nitrogeN cycle genes and soil chemical attributes. Forward selection (FS) and Monte Carlo permutation test were applied with 1000 random permutations to verify the significance of soil chemical properties on N cycle gene variation. RDA plots were generated using Canoco 4.5 (Biometrics, Wageningen, the Netherlands). We used the two-way PERMANOVA (Anderson, 2005) to group the treatments for similarity. The heatmap was performed calculating the Spearman correlation coefficients ($p \leq 0.05$) to evaluate the relationship between relative abundance of N cycle functional genes and soil chemical properties.

4.3 RESULTS

4.3.1 Soil chemical properties

The greatest change in soil chemical properties was observed in the liming treatment, followed by the lime + gypsum treatment (Table 1). The most notable effect of gypsum application on soil properties was an increase in soil S-SO_4^{2-} levels. Lime increased SOM; reduced soil acidity (pH) and aluminum toxicity; increased exchangeable K^+, Ca^{2+} and Mg^{2+} and, consequently, exchangeable bases (EB), BS and CEC; improved the availability of P and S-SO_4^{2-}; and increased NO_3^- and total inorganic nitrogen. However, liming with or without gypsum reduced the availability of cationic micronutrients (Fe, Mn, Cu and Zn) in the soil.

Table 1. Soil chemical properties

Soil chemical properties†	units	Control	Gypsum	Lime	Lime + Gypsum	*p value*
pH ($CaCl_2$)	-	3.75 c	4.15 b	5.99 a	6.15 a	0.001
SOM	g kg^{-1}	28.6 b	30.1 b	33.4 a	34.7 a	0.002
NO_3^-	mg kg^{-1}	22.9 c	24.5 c	30.0 b	32.5 a	0.001
NH_4^+	mg kg^{-1}	20.7 b	18.3 b	29.6 ab	35.1 a	0.041
N_i	mg kg^{-1}	43.5 b	42.8 b	59.7 a	67.6 a	0.001
$P_{(resin)}$	mg kg^{-1}	22.9 c	39.9 b	48.0 b	63.7 a	0.001
K^+	$mmol_c$ kg^{-1}	3.84 a	3.57 a	3.55 a	3.01 a	0.079
Ca^{2+}	$mmol_c$ kg^{-1}	13.3 c	28.1 b	66.6 ab	81.8 a	0.001
Mg^{2+}	$mmol_c$ kg^{-1}	6.72 c	4.88 c	34.1 a	28.3 b	0.001
$S\text{-}SO_4^{2-}$	mg kg^{-1}	12.2 b	22.8 a	14.5 b	17.3 ab	0.002
Al^{3+}	$mmol_c$ kg^{-1}	10.3 a	8.41 a	0.00 b	0.00 b	0.001
H+Al	$mmol_c$ kg^{-1}	65.5 a	61.8 a	21.2 b	20.0 b	0.001
EB	$mmol_c$ kg^{-1}	23.9 c	36.6 b	104 a	113 a	0.001
CEC	$mmol_c$ kg^{-1}	89.4 b	98.4 b	125 a	133 a	0.003
BS	%	26.7 c	37.2 b	83.1 a	84.9 a	0.001
Fe	mg kg^{-1}	33.9 a	31.7 a	29.3 ab	27.1 b	0.040
Mn	mg kg^{-1}	22.0 a	12.7 b	11.4 bc	9.47 c	0.001
Cu	mg kg^{-1}	3.76 a	2.46 b	2.15 b	1.95 b	0.005
Zn	mg kg^{-1}	1.36 a	1.26 a	1.00 b	1.00 b	0.002

Different lower-case letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. †Soil organic matter (SOM), nitrate (NO^{3-}), ammonium (NH_4^+), total inorganic nitrogen (N_i), phosphorus available (P_{resin}), exchangeable potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), sulfur-sulfate ($S\text{-}SO_4^{2-}$), exchangeable Al^{3+}, potential acidity (H+Al), sum of exchangeable bases (EB), cation exchange capacity (CEC), base saturation (BS) and available iron (Fe), manganese (Mn), cupper (Cu) and zinc (Zn).

4.3.2 N cycle functional genes (real-time PCR)

Quantitative PCR based on the 16S rRNA gene was used to estimate the total abundance of archaea and bacteria in bulk soil and in the rhizospheres of maize and ruzigrass cultivated in soils under different treatments (control, gypsum, lime and lime + gypsum) (Fig. 2). The total archaeal abundance was higher in the rhizospheres of maize ($p < 0.001$) and ruzigrass ($p < 0.001$) in the lime and lime + gypsum treatments than in the gypsum and control treatments (Fig. 2A, 2B). Similarly, in the bulk soil samples, the total archaeal abundance was higher ($p < 0.001$) in the lime and lime + gypsum treatments than in the gypsum and control treatments (Fig. 2C). For total bacteria, higher abundances were observed in the rhizospheres (Fig. 2D, 2E) than in the bulk soil (Fig. 2F). The total bacterial abundance in the ruzigrass rhizosphere was highest ($p < 0.001$) in the lime + gypsum treatment followed by the lime and gypsum treatments (Fig. 2E).

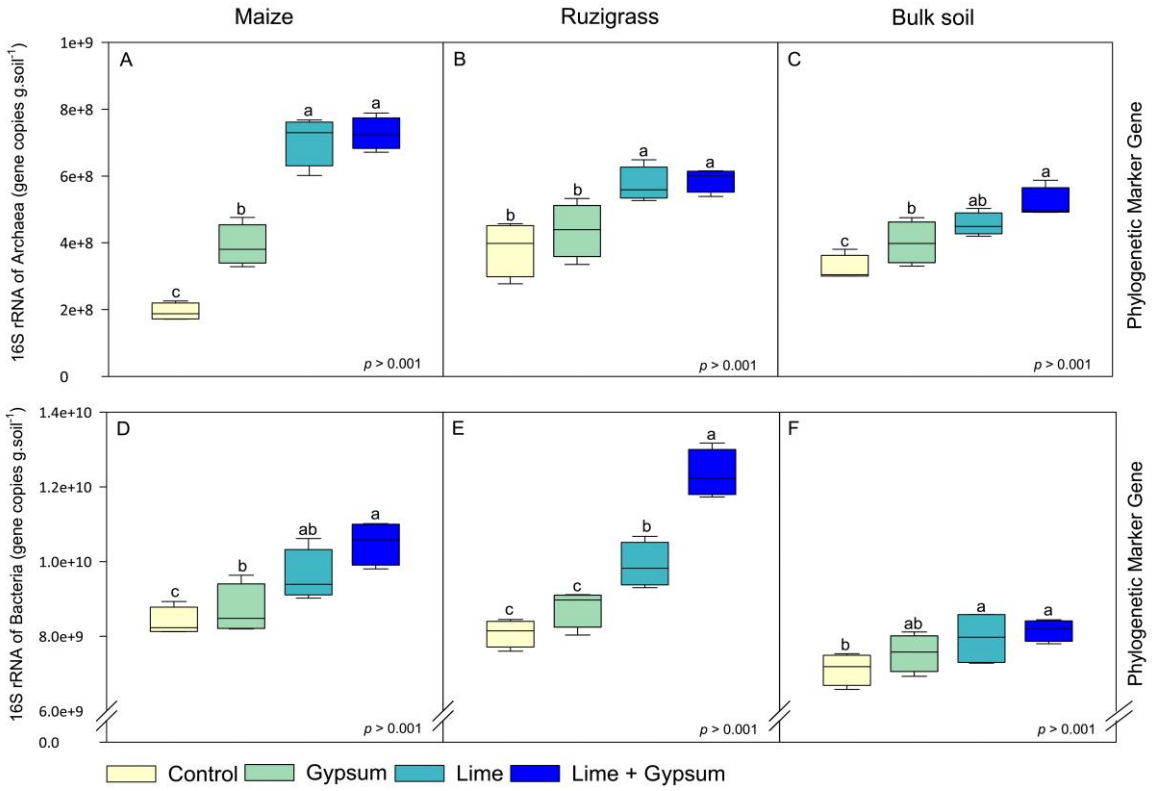


Figure 2. Archaeal and bacterial *16S rRNA* copy numbers in the rhizospheres of maize (A, D) and ruzigrass (B, E) and in bulk soil (C, F) under different treatments (control, gypsum, lime and lime + gypsum). Different lowercase letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 12$).

The lime + gypsum application resulted in the greatest increase in *nifH* copy numbers compared with the control (Supplementary Fig. S2). The *nifH* copy number increased significantly ($p < 0.001$) in the rhizospheres of both plants (Supplementary Fig. S2A and S2B) under the different treatments and was highest in the lime + gypsum treatment, followed by the lime and gypsum treatments. In bulk soil, the abundance of *nifH* was highest ($p < 0.001$) in the lime and lime + gypsum treatments (Supplementary Fig. S2C)

The abundances of *amoA* archaeal (AOA) and *amoA* bacterial (AOB) genes in the maize rhizosphere increased ($p < 0.001$) in the treatments compared with the control, and the abundances of both genes were highest in the lime + gypsum treatment (Supplementary Fig. S2D and S2G). By contrast, in the ruzigrass rhizosphere, the abundances of AOA ($p < 0.001$) and AOB ($p < 0.001$) decreased in the lime + gypsum treatment. In bulk soil, the abundances of AOA ($p < 0.001$) were the same regardless of treatment (Supplementary Fig. S2F), while the abundances of AOB were lower ($p <$

0.001) in the lime and lime + gypsum treatments than in the control and gypsum treatments (Supplementary Fig. S2I).

The copy numbers of *nirK* and *nosZ*, genes related to the denitrification process, in the maize rhizosphere did not differ (p = 0.544; p = 0.682) among the treatments and the control (Supplementary Fig. S2J and S2M). However, in the ruzigrass rhizosphere, the abundances of these two genes were lower (p < 0.001) in the lime and lime + gypsum treatments than in the control treatment (Supplementary Fig. S2K and S2N). By contrast, in bulk soil, the *nirK* (p < 0.001) and *nosZ* (p < 0.001) abundances showed similar decreasing patterns from the control to the gypsum, lime and lime + gypsum treatments (Supplementary Fig. S2L and S2O). In general, the increase in the abundance of phylogenetic marker genes correlated positively with the abundance of genes related to the N cycle (Supplementary Fig. S3). On the other hand, *nifH* was negatively correlated with denitrification genes.

Interestingly, by calculating the relative abundances (ratio of N cycle gene copy numbers to 16S rRNA gene (bacterial or archaeal) copy numbers), we found that the relative abundance of *nifH* in the maize and ruzigrass rhizospheres and in bulk soil increased (p < 0.001) successively from the control to the gypsum, lime and lime + gypsum treatments (Fig. 3A-3C). The increases in *nifH* abundance were higher in both plant rhizospheres than in bulk soil. Opposite results were obtained (p < 0.001) for the copy numbers of genes related to nitrification (*amoA* AOA and *amoA* AOB; Fig. 3D-3I) and denitrification (*nirK* and *nosZ*; Fig. 3J-3O), which showed similar decreasing patterns from the control to the gypsum, lime, and lime + gypsum treatments. The decreases in the relative abundances of these genes due to liming treatment were observed in both rhizospheres and bulk soil.

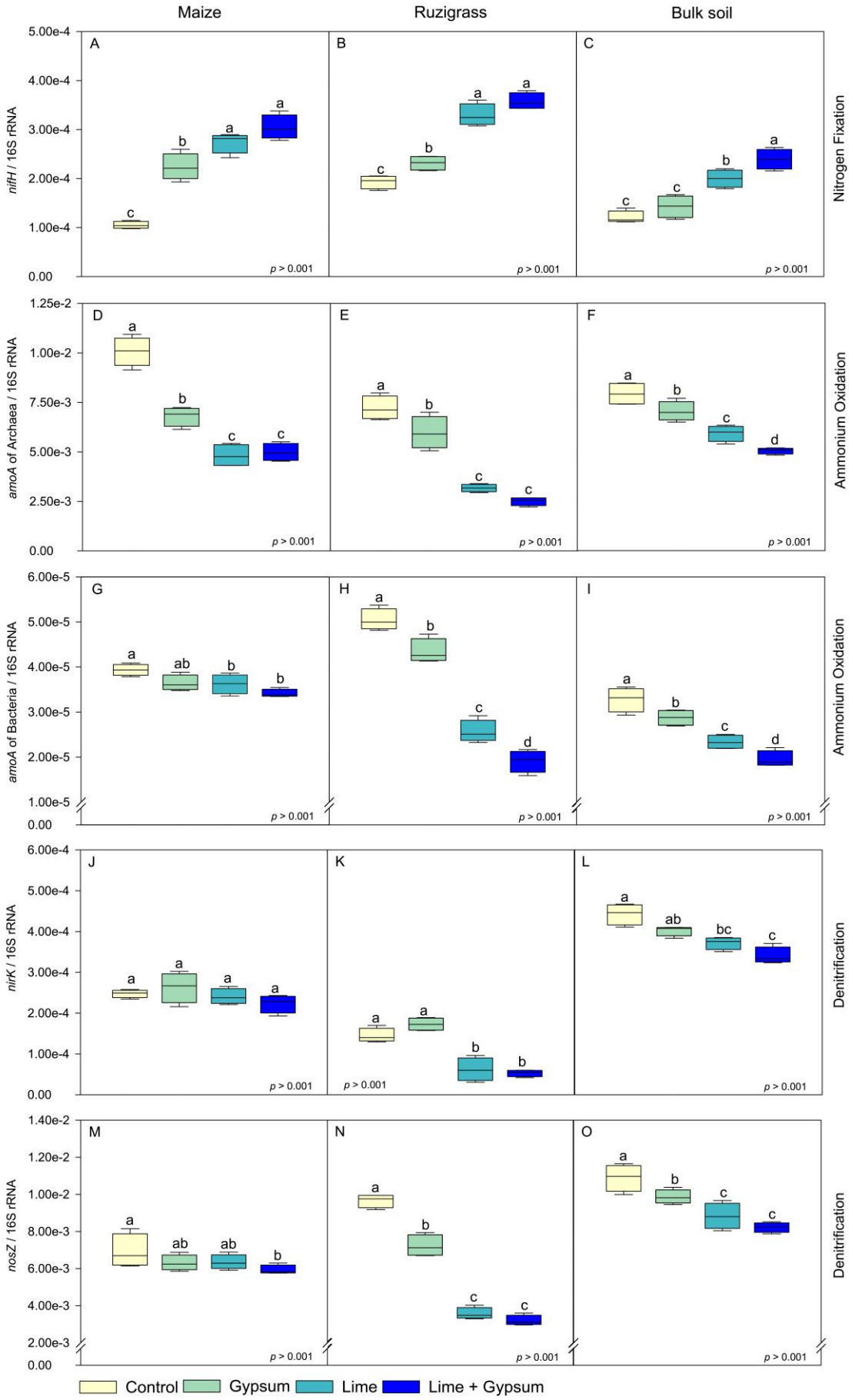


Figure 3. Ratio of the copy number of N cycle functional genes to the copy number of

archaeal or bacterial *16S rRNA* in the rhizospheres of maize (A, D, G, J, M) and ruzigrass (B, E, H, K, N) and in bulk soil (C, F, I, L and O) under different treatments (control, gypsum, lime and lime + gypsum). Different lowercase letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 12$).

4.3.3 Redundancy analysis (RDA) and correlation between the relative abundance of N cycle functional genes and soil chemical properties

Redundancy analysis (RDA) was performed to evaluate the soil chemical properties and the relative abundance of N cycle genes in the maize (Fig. 4A) and ruzigrass (Fig. 4B) rhizospheres and in bulk soil (Fig. 4C). The sum of the first and second axes explained 90.9% of the total variation of N cycle gene abundance in the maize rhizosphere (Fig. 4A). Axis 1 explained 85.6% of the separation of N cycle gene abundance into 3 main groups (PERMANOVA $F = 27.61$, $p < 0.001$): group 1, control; group 2, gypsum; and group 3, lime and lime + gypsum (Fig. 4A). The gene abundances in the maize rhizosphere in the control treatment were significantly different ($p < 0.05$) from those in the gypsum, lime and lime + gypsum treatments (Fig. 4A). Group 2, which comprised the gypsum-only treatment, showed intermediate behavior between groups 1 and 3. In group 3, the N cycle gene abundance in the maize rhizosphere was not significantly different between the lime and lime + gypsum treatments. According to RDA analysis followed by Monte Carlo permutation, N cycle gene abundance showed significant correlations with Ca^{2+} ($F = 78.95$; $p < 0.001$), Mg^{2+} ($F = 3.92$; $p = 0.027$) and Mn ($F = 5.38$; $p = 0.033$). Ca^{2+} and Mg^{2+} availability was related to *nifH* abundance, while Mn was related to the abundances of AOA, AOB, *nirK* and *nosZ* (Fig. 4A).

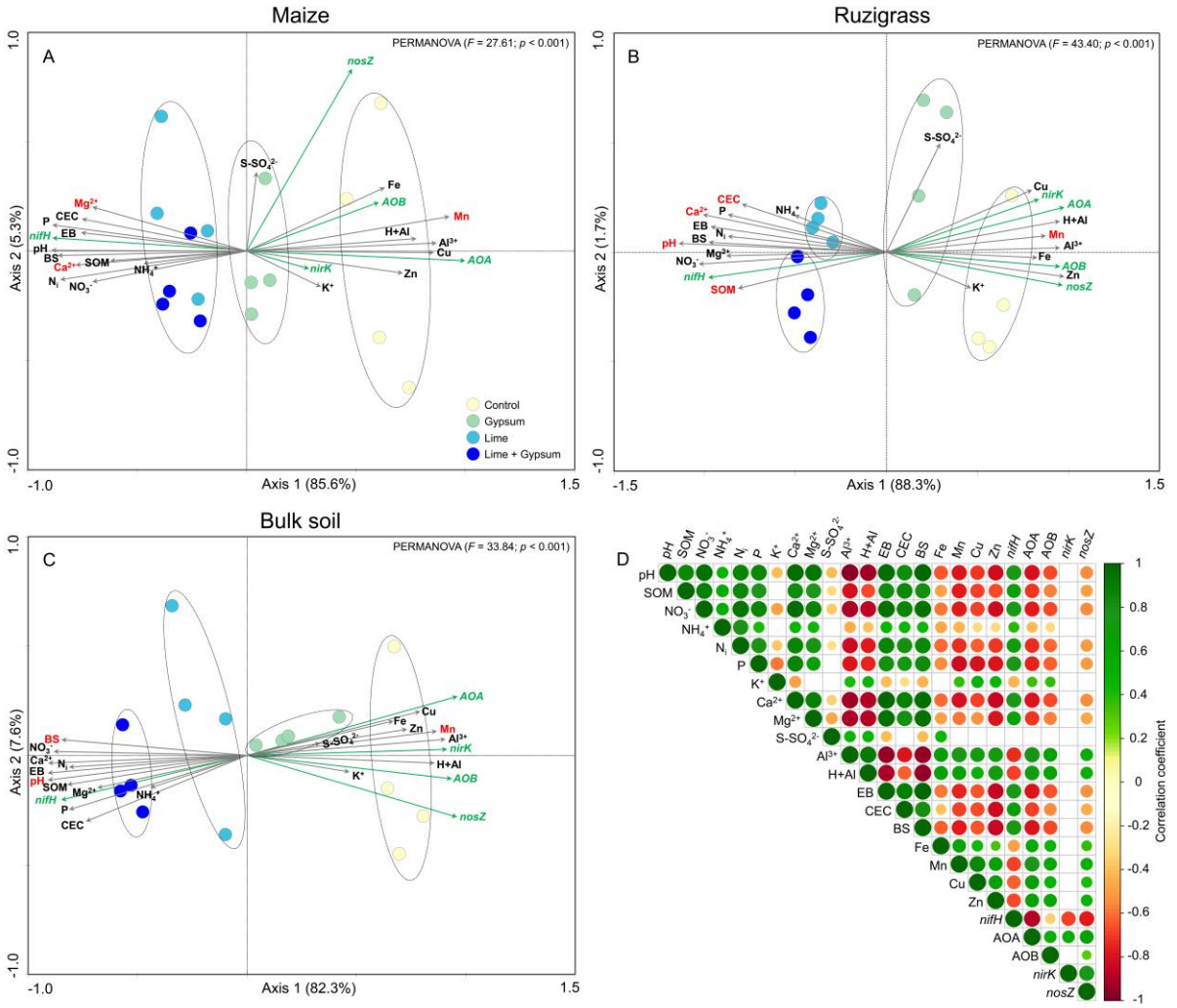


Figure 4. Redundancy analysis (RDA) of the relative abundance of N cycle functional genes and soil chemical properties (A-C). The arrows indicate correlations between factors. The significance of these correlations was evaluated by a Monte Carlo permutation test and is indicated by red color ($p \leq 0.05$). The dashed lines indicate significant clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). Heatmap of the correlation coefficients (Spearman) among the relative abundance of N cycle functional genes and soil chemical properties (D). Only significant correlations at $p \leq 0.05$ are shown.

In the ruzigrass rhizosphere, soil factors explained 90% of the N cycle gene abundance variation (Fig. 4B). Axis 1 explained 88.3% of the separation among the control and gypsum, lime, and lime + gypsum. In contrast to the maize rhizosphere (Fig. 4A), the ruzigrass rhizosphere samples were separated into four distinct groups by PERMANOVA analysis ($p < 0.001$), representing the four different treatments. The abundance of N cycle genes in the ruzigrass rhizosphere in the control treatment was significantly different ($p < 0.05$) from those in the gypsum, lime and lime + gypsum treatments (Fig. 4B). Furthermore, Monte Carlo permutation analysis showed

significant correlations of ruzigrass rhizosphere N cycle gene abundance with pH (F = 237; $p < 0.001$), SOM (F = 6.42; p = 0.008), Ca^{2+} (F = 6.79; p = 0.004), CEC (F = 3.21; p = 0.049) and Mn availability (F = 3.67; p = 0.044). pH, SOM, exchangeable Ca^{2+} and CEC were correlated with *nifH* abundance, whereas Mn availability was mostly related to AOA, AOB, *nirK* and *nosZ* abundance (Fig. 4B).

PERMANOVA analysis segregated the N cycle gene abundance in bulk soil into four groups ($p < 0.001$) (Fig. 3C), similar to the ruzigrass rhizosphere (Fig. 4B). Soil chemistry was responsible for 89.9% of the variation in N cycle gene abundance (Fig. 4C). Monte Carlo permutation analysis showed significant correlations of N cycle gene abundance with pH (F = 57.2; $p < 0.001$), K^+ (F = 5.83; p = 0.032) and BS (F = 4.72; p = 0.043). pH and BS were related to *nifH* gene abundance, and Mn availability was related to AOA, AOB, *nirK* and *nosZ* abundance.

Correlation analysis of N cycle gene abundance with soil chemical factors showed that the improvement in soil chemical properties due to lime and/or gypsum amendment increased the abundances of genes related to the N cycle (Fig. 4D). Increases in SOM, inorganic N, P, Ca^{2+}, Mg^{2+} and consequently EB, CEC and BS were negatively correlated with Al^{3+}, H+Al, Fe, Mn, Cu and Zn availability (Fig. 4D).

4.3.4 N content in maize and ruzigrass leaves and maize grain yield

The maize (Fig. 5A) and ruzigrass (Fig. 5B) leaf nitrogen content and maize grain yield (Fig. 5C) increased under the individual amendments compared with the control, but joint application of lime and gypsum provided the largest increases.

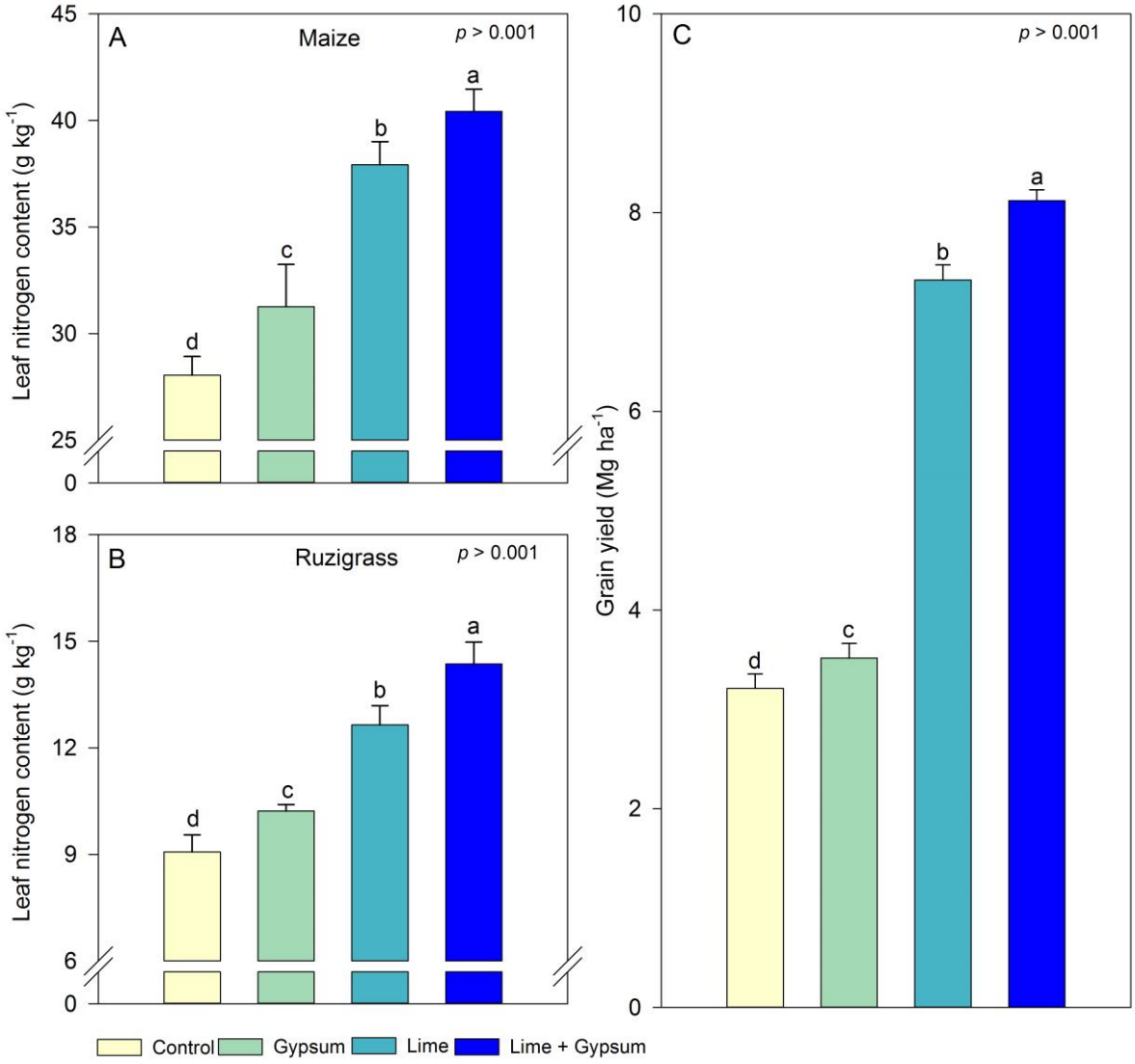


Figure 5. Maize and ruzigrass leaf nitrogen content (A and B) and maize grain yield (C) under different treatments (control, gypsum, lime and lime + gypsum). Different lowercase letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

4.4 DISCUSSION

4.4.1 Changes in soil chemical properties

In general, our results showed lasting effects for at least three years after exclusive liming application in 2002, 2004, 2010 and 2016, with improvements in all soil chemical attributes, including reduced pH, exchangeable Al^{3+}, H+Al and micronutrients (Fe, Mn, Cu and Zn) and greatly increased SOM, inorganic N, P, Ca^{2+} and Mg^{2+} availability and consequently CEC and BS compared with the control

treatment (Table 1). Thus, liming triggers soil buffering processes that change the dynamics of exchangeable cations and the dissolution of other elements. Moreover, as pH increases, the availability of metal ions such as Al^{3+} and micronutrients decreases, reducing possible toxic effects on plant development and microbial growth (Caires et al., 2011; Carmeis Filho et al., 2017). Therefore, the liming process has complex impacts on soil, which are reflected in a series of dependent and simultaneous effects and subsequent changes to soil processes (Holland et al., 2018).

Exclusive gypsum application resulted in a small increase in soil properties compared with the control treatment (Table 1), in agreement with Caires et al. (2011) and Zoca and Penn (2017). Although gypsum does not directly affect soil pH, it increases P, Ca^{2+}, S-SO_4^{2-} and CEC availability and reduces Mn and Cu availability. These changes in turn increase pH due to ligand exchange reactions of S-SO_4^{2-} with terminal hydroxides associated with Al and Fe oxides, which displace OH^- and promote partial neutralization of soil acidity. The improvements in crop yield due to gypsum application are primarily due to the increased Ca^{2+} and S solubility in soil and, consequently, availability in plants and/or reduced Al^{3+} availability in soil, mainly in deep layers (Soratto et al., 2010). Due to the thermodynamics of ion exchange and the properties of Ca^{2+}, gypsum can also potentially increase leaching losses of Mg^{2+} and K^+ to deep layers (Syed-Omar and Sumner, 1991; Zoca an Penn, 2017).

When applied separately, both lime and gypsum amendment improved soil fertility in our study, but the effects of lime were greater than those of gypsum, indicating the impossibility of replacing lime with gypsum in agricultural systems. However, the effects of joint application of lime and gypsum were greater than those of either amendment individually, particularly for N, P, Ca^{2+} and S-SO_4^{2-} availability. The availability of other soil factors remained similar to those obtained by lime application only, particularly Mg^{2+} availability. Several studies have demonstrated that the effects of gypsum on soil chemical properties are smaller than those of lime (Zoca and Penn, 2017, Bossolani et al., 2018, Crusciol et al., 2019), mainly because gypsum is not an acid-neutralizing or acid-forming substance (Zoca and Penn, 2017). Thus, liming has the greatest effects on soil, but our work demonstrates that gypsum is an important complement to lime application that potentiates its effects, especially under tropical conditions with intensive cropping.

4.4.2 N cycle genes in an intercropped system with soil amendment

The addition of lime and/or gypsum to soil in the no-till intercropping system changed the soil chemical properties and consequently had positive effects on total archaea, total bacteria and N cycle genes in the bulk soil and rhizospheres of both plant species evaluated. Gypsum increased total archaea and total bacteria, but liming produced the largest increases in these groups in plant rhizospheres and soil.

The responses of N cycle genes in the rhizosphere to amendment differed between maize and ruzigrass, even though both plant species were cultivated under the same soil conditions with intercropping. Plant species can alter rhizosphere characteristics (for example, pH and root exudates) (Chen et al., 2019), and these alterations support the formation of a microhabitat (Schmidt et al., 2018) that can be occupied by different amounts and species of microorganisms specialized in various niches (Suleiman et al., 2019). The treatments with lime and/or gypsum changed soil nutrient availability, and these changes interacted strongly with the changes in the crop system induced by the plants themselves, further contributing to shaping the microbial community in these agroecosystems (Navarrete et al., 2013).

The abundances of N cycle genes increased proportionally to population growth of archaea and bacteria (Supplementary Fig. S2 and S3), but the responses differed between the plant rhizospheres and bulk soil. Thus, the relative abundances of N cycle genes in relation to the total population of archaea and bacteria were examined to determine the real contribution of these genes in the microbial community according to the treatments employed (Fig.3). The relative abundance of *nifH* increased in all treatments compared with the control; however, exclusive liming and lime + gypsum amendment enhanced the abundance of this gene in the soil and plant rhizospheres (Supplementary Fig. S2A-S2C). Likewise, the relative abundance of *nifH* in relation to the total population of bacteria increased under the application of lime exclusively or lime + gypsum, indicating a high contribution of these amendments to the nitrogen-fixing community in the soil. There was a strong correlation between soil factors and the relative abundance of *nifH* (Fig. 4D), indicating that the improvement in soil attributes (e.g., increases in pH and availability of Ca^{2+} and Mg^{2+} and reduced Al^{3+}) increased nitrogen fixers in the microbial community. RDA enabled the identification of the most important soil factors for increasing the relative abundance of the *nifH* gene in each compartment evaluated in the crop system (maize and ruzigrass rhizospheres

and bulk soil; Fig. 4A-4C). Different species of plants vary in their recruitment of microorganisms, mainly due to differences in root exudates (Kuzyakov and Razavi, 2019). *nifH* abundance is completely dependent on soil fertility and how each amendment changes soil factors. In this study, nitrogen fixers responded significantly to pH and base saturation, but the responses in the rhizospheres of intercropped maize and ruzigrass differed. Exchangeable Ca^{2+} and Mg^{2+} were the most important soil factors shaping the relative abundance of *nifH* in the maize rhizosphere, whereas in the ruzigrass rhizosphere, SOM, pH, exchangeable Ca^{2+} and CEC provided the greatest changes in *nifH* abundance.

The low pH range in the control and gypsum treatments (Table 1) showed high availability of Al^{3+}, which could be highly toxic to N fixers (Mokolobate and Haynes, 2002; Jaiswal et al., 2018). Mg^{2+} is an essential cofactor for nitrogenase function and assists in electron transfer (Mortenson et al., 1973). Soil factors, mainly Ca^{2+} and Mg^{2+}, have been frequently reported to increase nitrogen fixer populations in different agroecosystems (Wakelin et al. 2010; Mirza et al. 2014; Wang et al., 2018) and might explain the increase in *nifH* in our study. During the summer, soybean is cultivated in the study system; the increase in the amount of nitrogen fixers in association with soybean may favor higher yields, since long-term nitrogen fertilization of this leguminous crop has not been carried out in the area. The high abundance of *nifH* may be associated with this summer crop, however, although soybean is inoculated with *Bradyrhizobium* sp. every crop season, the amount of inoculant used is the same in all treatments. Hence, the difference in the abundance of *nifH* obtained is attributed exclusively to the effect of the treatments. The abundances of ammonia-oxidizing communities of both archaea (AOA) and bacteria (AOB) were modified by the application of different amendments, but distinct patterns were observed between the plant rhizospheres and bulk soil (Supplementary Fig. S2D-S2I). In the maize rhizosphere, AOA and AOB increased, whereas in the ruzigrass rhizosphere and bulk soil they decreased. Our data indicate a strong capacity of forage grasses to reduce soil nitrification compared with maize. The improvement of soil components may contribute to reducing soil nitrification, as evidenced by the reduction in the abundance of AOB in bulk soil. Interestingly, we observed decreases in the relative abundances of AOA and AOB in the soil regardless of plant species (maize or ruzigrass), and thus improving soil chemistry through lime and gypsum amendments can reduce the nitrification process in intercropping systems under no-till.

Soil factors that were positively correlated with the increases in the relative abundances of nitrifier and denitrifier genes were identified by RDA (Fig. 4A-4C) and further supported by correlation analysis (Fig. 4D). In general, high levels of micronutrients (mainly Mn), Al^{3+} and H+Al were associated with greater abundances of AOA, AOB, *nirK* and *nosZ*. Exclusive liming and liming + gypsum reduced the abundances of *nirK* and *nosZ* in the ruzigrass rhizosphere and bulk soil but not in the maize rhizosphere (Supplementary Fig. S2J-S2O). The relative abundances of these genes exhibited the same patterns (Fig. 3J-3O), and in general, the improvement of soil attributes correlated negatively with nitrifier and denitrifier gene abundance (Fig. 4D). Thus, our results demonstrate that soil chemistry is an important factor linked to the abundance of genes related to nitrification and denitrification and that micronutrients are important factors in these processes.

Generally, acidic environments favor the availability of micronutrients, which can be toxic to plants and microorganisms; thus, soil acidity correction is an important management strategy to balance the availability of these micronutrients and reduce the abundances of nitrifiers and denitrifiers (Liu et al., 2010). Almost every enzymatic pathway in the N cycle involves a metallic cofactor containing Fe, Cu or sometimes Zn (Godfrey and Glass, 2011). In addition, in tropical soils with low SOM, oxidation of NH_4^+ can occur in the presence of metal oxides like manganese oxides (Swathi at al., 2017), which explains the strong influence of Mn in our results (Fig. 4A-4C). This element plays a major role in biogeochemical nitrogen cycling (Hulth et al., 1999). MnO_x acts as a terminal electron acceptor (Lin and Taillefert, 2014) and mediates the reduction of NH_4^+. Bru et al. (2011) and Levy-Booth et al. (2014) reported that in addition to pH, Mn levels were positively correlated with the abundances of *nirK*, *nirS* and *nosZ* genes. Ligi et al. (2014) demonstrated that *nirK* and *nosZ* gene abundances are most similarly influenced by the same environmental parameters.

Changes in bacterial community composition have been shown to influence the nitrogen cycling potential of soils (Wakelin et al., 2009). These observations suggest that liming, regardless of gypsum addition in the present research, is an important strategy for efficiently reducing overall N_2O emissions through nitrification and denitrification in crop systems that utilize nitrogen fertilizers, although different responses are found in several other greenhouse gases (Holland et al., 2018). An extensive review of the impact of liming on greenhouse gas emissions, Holland et al. (2018) and Kunhikrishnan et al. (2016) showed that impacts on N transformation due

to liming include increased CO_2 flux and decreased of oxidation rates of CH_4 and N_2O emissions. Quantification of liming effect showed that there was four times decreased N_2O emissions from a limed soil (pH 7.0) compared to a soil with pH 4.5 (Baggs et al., 2010). Another key gaseous process is volatilization, that has been shown to increase by liming, which raises NH_3 emissions (Sommer and Ersbøll, 1996). In terms of the direct net liming impact on gases flux there is evidence that liming is not a sound mitigation strategy (Gibbons et al., 2014) and thus, overall greenhouse gas emissions are increased after liming. According to Holland et al. (2018), the impacts of liming on greenhouse gas emissions are complex and there are markedly different potential changes in emissions between different gases.

Despite the vast literature indicating that pH is the greatest determinant of microbial assembly (Bartram et al. 2014; Lammel et al., 2018; Holland et al., 2018), this study clearly shows that the increase in soil pH due to liming directly impacts many other soil factors that in turn directly affect microbial enzymes related to the N cycle, i.e., EB, CEC, BS, Al^{3+} and micronutrient availability. However, most studies of the N cycle in agricultural soils and the soil microbial community do not consider soil chemical factors and attach importance only to pH and SOM (Zhang et al., 2017; Liu et al., 2018; Holland et al., 2018). Thus, the development of sustainable crop systems in tropical regions is necessary to improve overall agricultural functionality (Moraes et al., 2019), as most tropical agricultural soils are acidic with low fertility.

4.4.3 N content in maize and ruzigrass leaves and maize grain yield

The high N concentration in maize and ruzigrass leaves (Fig. 4A and 4B) may be a consequence of the higher N availability in the soil amended with lime + gypsum (Table 1). Soil ameliorants such as lime can increase biological processes that govern the activity of soil microorganisms responsible for SOM mineralization (Kemmitt et al., 2006; Stiehl-Braun et al., 2011; Wachendorf, 2015). The responses to liming vary according to the crop system and whether long-term or recent effects are examined. The positive effects of liming on soil fertility induce changes in soil properties and can promote cascade effects on soil N transformation processes (Holland et al., 2018). Several studies have associated liming with increased nitrification and denitrification in subtropical environments, but studies in tropical soils like the current study are rare. The research shows that repeated liming applications will increase soil N

mineralization (Holland et al., 2018), but the overall impact of liming will depend on immobilization that occur based on the C:N ratio of plant residues returned to soils and quality of the organic matter (Bailey, 1995; Kliemman et al., 2006; Wachendorf, 2015). Nitrogen is considered the nutrient that better returns to the soil-plant system through plant residues and can be reused by the crops in subsequent agricultural cycles (Hungria et al., 2015).

Other factors must also be considered. (a) Exclusive liming or, in particular, gypsum addition increases the depth and volume of root system exploration (Costa et al., 2018), which reduces losses of N by leaching. (b) Plants growing in chemically fertile environments are more vigorous and can compete with microorganisms for soil resources, mainly N, which reduces the availability of N for nitrification and subsequent denitrification processes. (c) Most studies reporting increased nitrification in amended soils do not consider losses due to denitrification or the possibility of reduced nitrification in intercropping systems with grain crops and tropical grasses. Soils cultivated with forage grasses reduce nitrifying activity. Consequently, the residual N in the soil, whether from top dressing fertilization in maize or from nutrient cycling, remains in the form of NH_4^+ and is no longer linked to soil CEC, thereby reducing losses by leaching and denitrification (Subbarao et al., 2015, Coskun et al., 2017).

Finally, our findings demonstrated that liming and lime + gypsum amendment affect the microbiota and soil chemical properties, thereby influencing maize development and grain yield (Fig. 5C) through resource availability. In addition, the increase in nitrogen absorption by plants increases the absorption and metabolism of other nutrients (Marschner, 2012), which is reflected in crop yield. Understanding how different cultivated plants respond to these changes is of paramount importance for developing more productive and sustainable agricultural systems. Therefore, the combination of lime and gypsum can be considered an important practice to improve the yield capacity of acidic tropical soils managed under no-till systems (Inagaki et al., 2016; Carmeis Filho et al., 2017).

4.5 CONCLUSIONS

Our results reveal how the lasting effects of long-term exclusive and joint application of lime and gypsum change soil chemical properties in a tropical intercropping no-till system and how these changes influence total prokaryotes

(archaea and bacteria) and genes related to the N cycle. These soil amendments increased soil fertility (mainly Ca^{2+} and Mg^{2+} status), reduced Al^{3+} availability and balanced the levels of soil micronutrients. The influences of these soil amendments on N cycle genes were similar in the rhizospheres of ruzigrass and maize and bulk soil, indicating that soil chemical properties provide strong selection of microorganisms and thus may be as relevant as the type of plant in intercropping systems. In addition, the improvement in soil quality led to greater N uptake by plants and higher maize yield. This study demonstrates that soil amendments are an important alternative for increasing the efficiency of nitrogen use in no-till agricultural systems and provides a basis for future research characterizing the active community and expression of functional genes of microorganisms related to the N cycle.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by São Paulo Research Foundation (FAPESP) (Grant 2018/11063-7 and 2019/12764-1), and The Netherlands Organization for Scientific Research (NWO) grant 870.15.022. In addition, the second and sixth authors would like to thank the National Council for Scientific and Technological Development (CNPq) for an award for excellence in research. Publication number 6978 of the Netherlands Institute of Ecology (NIOO-KNAW).

REFERENCES

Allen, V.G., Baker, M.T., Segarra, E., Brown, C.P., 2007. Integrated irrigated crop–livestock system in dry climates. Agronomy Journal 99, 346–360

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Moraes, G., Leonardo, J., Sparovek, G., 2013.

Anderson, M.J., 2005. Permutational multivariate analysis of variance. Department of Statistics, University of Auckland, Auckland, 26, 32-46.

Bardsley CE, Lancaster JD (1960) Determination of reserve sulfur and soluble sulfates in soils. Soil Sci Soc Am J 24:265–268. https://doi.org/https://doi.org/10.2136/sssaj1960.03615995002400040015x

Bartram, A.K., Jiang, X., Lynch, M.D., Masella, A.P., Nicol, G.W., Dushoff, J., Neufeld, J.D., 2014. Exploring links between pH and bacterial community composition in soils from the Craibstone Experimental Farm. FEMS microbiology ecology 87 (2), 403-415.

Baggs, E.M., Smales, C.L., Bateman, E.J., 2010. Changing pH shifts the microbial source as well as the magnitude of N2O emission from soil. Biology and Fertility of Soils 46, 793–805.

Bossolani, J.W., Lazarini, E., Santos, F.L., Sanches, I.R., Meneghette, H.H.A., Parra, L.F., Souza, L.G.M., 2018. Surface reapplication of lime and gypsum on maize cultivated sole and intercropped with Urochloa. Communications in Soil Science and Plant Analysis, 49 (15), 1855-1868.

Brody, J.R., Kern, S.E., 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques 36 (2), 214-217.

Bru, D., Ramette, R., Saby, N.P.A., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D., Philippot, L., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. The ISME Journal 5, 532-542.

Burton, J., Chen, C., Xu, Z., Ghadiri, H., 2007. Gross nitrogen transformations in adjacent native and plantation forests of subtropical Australia. Soil Biology & Biochemistry 39, 426-433.

Caires, E.F., Guimarães, A.M., 2018. A Novel Phosphogypsum Application Recommendation Method under Continuous No-Till Management in Brazil. Agronomy Journal 110 (5), 1987-1995.

Caires, E.F., Joris, H.A.W., Churka, S., 2011. Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. Soil Use and Management 27 (1), 45-53.

Cantarella, H., van Raij, B., Camargo, C.E.O., 1997. Cereals. In: van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C. (eds). Fertilization and liming recommendation for the state of São Paulo, Campinas, pp. 68-69.

Carmeis Filho, A.C., Penn, C.J., Crusciol, C.A., Calonego, J.C., 2017. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. Agriculture, Ecosystems & Environment 241, 11-23.

Chen, S., Waghmode, T.R., Sun, R., Kuramae, E., Hu, C., Liu, B., 2019. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome*, *7* (1), 1-13.

Clemensson-Lindell, A., Persson, H., 1992. Effects of freezing on rhizosphere and root nutrient content using two soil sampling methods. Plant and Soil 139, 39–45.

Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. How plant root exudates shape the nitrogeN cycle. Trends in Plant Science 22 (8), 661-673.

Costa, C.H., Carmeis Filho, A.C., Crusciol, C.A.C., Soratto, R.P., Guimarães, T.M., 2018. Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. Crop and Pasture Science 69 (5), 488-505.

Costa, C.H.M., Crusciol, C.A.C., 2016. Long-term effects of lime and phosphogypsumapplication on tropical no-till soybean–oat–sorghum rotation and soil chemical properties. European Journal of Agronomy 74, 119-132.

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutrient Cycling in Agroecosystems 115, 1-26.

DeAngelis, K.M., Brodie, E.L., DeSantis, T.Z., Andersen, G.L., Lindow, S.E., Firestone, M.K., 2009. Selective progressive response of soil microbial community to wild oat roots. The Isme Journal 3 (2), 168–178.

Food and Agriculture Organization - FAO, 2015. Status of the World's Soil Resources (SWSR)—Main report. Natural Resources and Environment Department, Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils (ITPS), Rome, Italy.

Franzluebbers, A.J., Gastal, F., 2019. Building Agricultural Resilience with Conservation Pasture-Crop Rotations. Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. p. 109–121.

Garcia, C.M.P., Costa, C., Andreotti, M., Meirelles, P.R.L., Pariz, C.M., Freitas, L.A., Teixeira Filho, M.C.M., 2016. Wet and dry maize yield under intercrop culivation with marandu grass and/or dwarf pigeon pea and nutritional value of the marandu grass in succession. Australian Journal of Crop Science 10, 1564–1571.

Gibbons, J.M., Williamson, J.C., Williams, A.P., Withers, P.J., Hockley, N., Harris, I.M., Hughes, J.W., Taylor, R.L., Jones, D.L., Healey, J.R., 2014. Sustainable nutrient management at field, farm and regional level: Soil testing, nutrient budgets and the trade-off between lime application and greenhouse gas emissions. Agriculture, Ecosystems & Environment 188, 48-56.

Godfrey, L.V., Glass, J.B., 2011. The geochemical record of the ancient nitrogeN cycle, nitrogen isotopes, and metal cofactors. Methods in enzymology 486, 483-506.

Guo, A., Ding, L., Tang, Z., Zhao, Z., Duan, G., 2019. Microbial response to $CaCO_3$ application in an acid soil in southern China. Journal of Environmental Sciences, 79, 321-329.

Heanes, D.L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Commun Soil Sci Plant Anal, 10, 191-1213.

Holland, J.A., Bennett, A., Newton, B., McKenzie, P., White, T., George, R., Pakeman, J.S., Bailey, D.A., Fornara, R. Hayes., 2018. Liming impacts on soils, crops and biodiversity in the UK: a review. Science of the Total Environment 610, 316-332.

Hulth, S., Aller, R.C., Gilbert, F., 1999. Coupled anoxic nitrification/manganese reduction in marine sediments. Geochimica et Cosmochimica Acta 63, 49-66.

Inagaki, T.M., de Moraes Sá, J.C., Caires, E.F., Gonçalves, D.R.P., 2016. Lime and gypsum application increase biological activity, carbon pools, and agronomic

productivity in highly weathered soil. Agriculture, Ecosystems & Environment 231, 156-165.

Jaiswal, S.K., Naamala, J., Dakora, F.D., 2018. Nature and mechanisms of aluminium toxicity, tolerance and amelioration in symbiotic legumes and rhizobia. Biology and Fertility of Soils 54 (3), 309-318.

Jarvis, S.C., 1984. The responses of two genotypes of white clover to addition of lime to an acid permanent grassland soil. Journal of the Science of Food and Agriculture 35, 1149–1158.

Kemmitt, S.J., Wright, D., Goulding, K.W.T., Jones, D.L., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry 38, 898–911.

Kiehl, E.J., 1979. Edaphology manual: soil-plant relationship. Agronômica Ceres. São Paulo, Brazil: Piracicaba; 264 p.

Kim, N., Zabaloy, M.C., Guan, K., Villamil, M.B., 2020. Do cover crops benefit soil microbiome? A meta-analysis of current research. Soil Biology and Biochemistry 142, 107701.

Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22, 711-728.

Kunhikrishnan, A., Thangarajan, R., Bolan, N., Xu, Y., Mandal, S., Gleeson, D., Seshadri, B., Zaman, M., Barton, L., Tang, C., 2016. Functional relationships of soil acidification, liming, and greenhouse gas flux. Advances in Agronomy 139, 1–71.

Kuzyakov, Y., Razavi, B.S., 2019. Rhizosphere size and shape: Temporal dynamics and spatial stationarity. Soil Biology and Biochemistry 135, 343-360.

Lammel, D.R., Barth, G., Ovaskainen, O., Cruz, L.M., Zanatta, J.A., Ryo, M., Souza, E. M., Pedrosa, F.O., 2018. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. Microbiome, 6 (1), 1-13.

Levy-Booth, D.J., Prescott, C.E., Grayston, S.J., 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biology and Biochemistry, 75, 11-25.

Li, X. G., Jousset, A., De Boer, W., Carriön, V. J., Zhang, T., Wang, X., Kuramae, E. E., 2019. Legacy of land use history determines reprogramming of plant physiology by soil microbiome. The *ISME Journal*, *13*, 738-751.

Ligi, T., Truu, M., Truu, J., Nõlvak, H., Kaasik, A., Mitsch, W.J., Mander, Ü., 2014. Effects of soil chemical characteristics and water regime on denitrification genes (nirS, nirK, and nosZ) abundances in a created riverine wetland complex. Ecological Engineering, 72, 47-55.

Lin, H., Taillefert, M., 2014. Key geochemical factors regulating Mn(IV)-catalyzed anaerobic nitrification in coastal marine sediments. Geochimica et Cosmochimica Acta 133, 17-33.

Liu, B., Mørkved, P.T., Frostegård, Å., Bakken, L.R., 2010. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS microbiology ecology 72 (3), 407-417.

Liu, X.Y., Rashti, M.R., Esfandbod, M., Powell, B., Chen, C.R., 2018. Liming improves soil microbial growth, but trash blanket placement increases labile carbon and nitrogen availability in a sugarcane soil of subtropical Australia. Soil Research, 56 (3), 235-243.

Malavolta, E., Vitti, G.C., Oliveira, A.S., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações, 2 eds. Piracicaba: Potafos.

Mateus, G.P., Crusciol, C.A.C., Pariz, C.M., Costa, N.R., Borghi, E., Costa, C., Martello, J.M., Castilhos, A.M., Franzluebbers, A.J., Cantarella, H., 2020. Corn intercropped with tropical perennial grasses as affected by sidedress nitrogen application rates. Nutrient Cycling in Agroecosystems 1, 1-22.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5 (1), 62–71.

Mirza, B.S., Potisap, C., Nüsslein, K., Bohannan, B.J., Rodrigues, J.L., 2014. Response of free-living nitrogen-fixing microorganisms to land use change in the Amazon rainforest. Applied and Environmental Microbiology 80, 281-288.

Mokolobate, M., Haynes, R., 2002. Comparative liming effect of four organic residues applied to an acid soil. Biology and Fertility of Soils 35, 79–85

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, R.M., 2019. Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive Monoculture Systems. Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality.

Mortenson, L.E., Zumft, W.G., Palmer, G., 1973. Electron paramagnetic resonance studies on nitrogenase III. Function of magnesium adenosine 5′-triphosphate and adenosine 5′-diphosphate in catalysis by nitrogenase. Biochimica et Biophysica Acta 292 (2), 422-435.

Mulvaney, R.L., 1996. Nitrogen—inorganic forms. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. SSSA Book Series 5. Soil Science Society of America and American Society of Agronomy, Madison, WI, USA, pp. 1123-1184.

Navarrete, A.A., Kuramae, E.E., De Hollander, M., Pijl, A.S., Van Veen, J.A., Tsai, S. M., 2013. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiology Ecology 83* (3), 607-621.

Nelson, L.S., 1998. The Anderson-Darling test for normality. Journal of Quality Technology 30 (3), 298.

Pang, Z., Tayyab, M., Kong, C., Hu, C., Zhu, Z., Wei, X., Yuan, Z., 2019. Liming Positively Modulates Microbial Community Composition and Function of Sugarcane Fields. Agronomy 9 (12), 808.

Pariz, C.M., Costa, C., Crusciol, C.A.C., Castilhos, A.M., Meirelles, P.R.L., Roça, R.O., Pinheiro, R.S.B., Kuwahara, F.A., Martello, J.M., Cavasano, F.A., Yasuoka, J.I., Sarto, J.R.W., Melo, V.F.P., Franzluebbers, A.J., 2017. Lamb production

responses to grass grazing in a companion crop system with corn silage and oversowing of yellow oat in a tropical region. Agricultural Systems 151, 1–11.

Quaggio, J.A., van Raij, B., 1997. Soil acidity correction. In: van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C. (Eds.), Fertilization and liming recommendation for the state of São Paulo, Instituto Agronômico, Campinas, pp. 14-19.

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1993. How a corn plant develops. Special Report nº48. Iowa State University of Science and Technology. Cooperative Extension Service. Iowa, EEUU.

Rosolem, C.A., Ritz, K., Cantarella, H., Galdos, M.V., Hawkesford, M.J., Whalley, W.R., Mooney, S.J. (2017). Enhanced plant rooting and crop system management for improved N use efficiency. Advances in Agronomy 146, 205–239.

Savian, J.V., Barth Neto, A., David, D.B., Bremm, C., Schons, R.M.T., Genro, T.C.M., Amaral, G.A., Gere, J., McManus, C.M., Bayer, C., Carvalho, P.C.F., 2014. Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: Implications for integrated crop–livestock system. Agriculture Ecosystem and Environment 190, 112–119.

Schlemper, T R., Leite, M.F.A., Reis Lucheta, A., Shimels, M., Bouwmeester, H.J., van Veen, J.A., Kuramae, E.E., 2017. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS Microbiology Ecology 93* (8), 1-11.

Shoemaker HE, McLean EO, Pratt PF (1961) Buffer Methods for Determining Lime Requirement of Soils With Appreciable Amounts of Extractable Aluminum. Soil Sci Soc Am J 25:274–277. https://doi.org/10.2136/sssaj1961.03615995002500040014x

Schmid, M. W., Hahl, T., van Moorsel, S. J., Wagg, C., De Deyn, G. B., & Schmid, B. (2018). Rhizosphere bacterial community composition depends on plant diversity legacy in soil and plant species identity. BioRxiv, 287235.

Sommer, S.G., Ersbøll, A.K., 1996. Effect of air flow rate, lime amendments, and chemical soil properties on the volatilization of ammonia from fertilizers applied to sandy soils. Biology and Fertility of Soils 21, 53–60.

Stiehl-Braun, P.A., Powlson, D.S., Poulton, P.R., Niklaus, P.A., 2011. Effects of N fertilizers and liming on the micro-scale distribution of soil methane assimilation in the longterm Park Grass experiment at Rothamsted. Soil Biology and Biochemistry 43, 1034-1041

Subbarao, G. V., Yoshihashi, T., Worthington, M., Nakahara, K., Ando, Y., Sahrawat, K. L., Idupulapati, M, R., Jean-Christophe, L., Masahiro, K., Hans-Joachim, B., 2015. Suppression of soil nitrification by plants. Plant Science 233, 155-164.

Suleiman, A.K.A., Harkes, P., van den Elsen, S., Holterman, M., Korthals, G. W., Helder, J., Kuramae, E.E., 2019. Organic amendment strengthens interkingdom associations in the soil and rhizosphere of barley (*Hordeum vulgare*). *Science of the Total Environment* 695, 1-16.

Swathi, D., Sabumon, P.C., Maliyekkal, S.M., 2017. Microbial mediated anoxic nitrification-denitrification in the presence of nanoscale oxides of manganese. International Biodeterioration & Biodegradation 119, 499-510.

Syed-Omar, S.R., Sumner, M.E., 1991. Effect of gypsum on soil potassium and magnesium status and growth of alfalfa. Communications in Soil Science and Plant Analysis 22, 2017–2028.

Unicamp – Campinas University, 2020. Center of Meteorological and Climatic Research Applied to Agriculture. Municipalities climate of São Paulo State. Botucatu. (accessed on 21 Dec 2019).

USDA, 2014. Keys to soil taxonomy.US Gov. Print. Office, Washington, DC, pp. 327-328.

Van Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Chemical Analysis for Evaluation of Fertility of Tropical Soils. Campinas, Brazil, Instituto Agronômico.

Von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant and soil 171 (1), 1-15.

Wachendorf, C., 2015. Effects of liming and mineral N on initial decomposition of soil organic matter and post harvest root residues of poplar. Geoderma 259, 243-250.

Wakelin, S.A., Gregg, A.L., Simpson, R.J., Li, G.D., Riley, I.T., McKay, A.C., 2009. Pasture management clearly affects soil microbial community structure and N-cycling bacteria. Pedobiologia 52, 237–251.

Wakelin, S.A., Gupta, V.V.S.R., Forrester, S.T., 2010. Regional and local factors affecting diversity, abundance and activity of free-living, N_2-fixing bacteria in Australian agricultural soils. Pedobiologia 53, 391–399.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37 (1), 29–38.

Wang, C., Zheng, M.M., Hu, A.Y., Zhu, C.Q., Shen, R.F., 2018. Diazotroph abundance and community composition in an acidic soil in response to aluminum-tolerant and aluminum-sensitive maize (*Zea mays* L.) cultivars under two nitrogen fertilizer forms. Plant and Soil 424 (2), 463-478.

Zhang, K., Chen, L., Li, Y., Brookes, P.C., Xu, J., Luo, Y., 2017. The effects of combinations of biochar, lime, and organic fertilizer on nitrification and nitrifiers. Biology and Fertility of Soils 53 (1), 77-87.

Zoca, S.M., Penn, C., 2017. An important tool with no instruction manual: a review of gypsum use in agriculture. Advances in Agronomy 144, 1-44.

SUPPLEMENTARY MATERIAL

Table S1. Crops growing and treatments application scheme during the experimental period (from 2002 to 2019).

Agricultural year	Season		Treatments[†]
	Summer crop	Autumn-Winter-Spring crop	
2002/2003	*Oriza sativa*	*Avena strigosa*	Lime: 2.7 Mg ha^{-1} (71% ECCE[‡]) Gypsum: 2.1 Mg ha^{-1}
2003/2004	*Phaseolus vulgaris*	*Avena strigosa*	-
2004/2005	*Arachis hypogaea*	*Avena sativa*	Lime: 2.0 Mg ha^{-1} (71% ECCE) Gypsum: 2.1 Mg ha^{-1}
2005/2006	*Arachis hypogaea*	*Avena sativa*	-
2006/2007	*Zea mays* intercropped with *Urochloa brizantha*	*Urochloa brizantha* (after *Zea mays* harvest)	-
2007/2008	*Zea mays* intercropped with *Urochloa brizantha*	*Urochloa brizantha* (after *Zea mays* harvest)	-
2008/2009	*Glycine max*	*Avena strigosa*	-
2009/2010	*Glycine max*	*Sorghum vulgare*	-
2010/2011	*Zea mays*	*Crambe abyssinica*	Lime: 2.0 Mg ha^{-1} (88% ECCE) Gypsum: 2.1 Mg ha^{-1}
2011/2012	*Zea mays*	*Crambe abyssinica*	-
2012/2013	*Pennisetum glaucum*	*Triticum aestivum*	-
2013/2014	*Phaseolus vulgaris*	*Pennisetum glaucum*	-
2014/2015	*Phaseolus vulgaris*	*Urochloa brizantha* (1st crop season)	-
2015/2016	*Urochloa brizantha* (cv. Marandu) (2nd crop season)	*Urochloa brizantha* (3rd crop season)	-
2016/2017	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	Lime: 13.04 Mg ha^{-1} (69% ECCE) Gypsum: 10 Mg ha^{-1}
2017/2018	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	-
2018/2019	*Glycine max*	*Zea mays* intercropped with *Urochloa ruziziensis*	-

[†]The reapplications in October 2004, 2010 and 2016 were performed when base saturation reached ≤ 50%.
[‡]ECCE: effective calcium carbonate equivalents

Table S2 - Description of primers, standards DNA and amplification conditions that were used in qPCR analysis

	Target Genes	*Primers*	*DSMZ* code†	Sequence (5'- 3')	Fragment size (pb)	Reference	Conditions of Amplification
Phylogenetic	*16S rRNA Bacteria*	Eub 338f Eub 518r	17167	ACTCCTACGGGAGGCAGC AG ATTACCGCGGCTGCTGG	180	Bakke et al. (2011)	95ºC - 10 min, 40 cycles, 94ºC - 15 s, 56ºC - 30 s e 72 ºC - 45 s
	16S Archaea	519F 915R	Environmental DNA‡	CAGCCGCCGCGGTAA GTGCTCCCCCGCCAATTCC T	397	Klindworth et al. (2013)	95ºC - 10 min, 40 cycles, 95ºC - 30 s, 57ºC - 30 s e 72 ºC - 50 s
Functional genes associated with the N cycle	*nifH*	-	17167	AAA GGY GGW ATC GGY AAR TCC ACC AC TTG TTS GCS GCR TAC ATS GCC ATC AT	457	Wallenstein and Vilgalys, (2005)	95ºC - 5 min, 40 cycles, 95 ºC - 30 s, 59ºC - 30 s e 72ºC - 1 min e 72º C - 1 min
	amoA of Bacteria	*amoB 1F amoB 2F*	28437	GGG GTT TCT ACT GGT GGT CCC CTC KGS AAA GCC TTC TTC	491	Rotthauwe et al. (1997)	95ºC - 10 min, 40 cycles, 95ºC - 45 s, 60ºC - 45 s e 72ºC - 45 s
	amoA of Archaea	*amoA1F amoA 2F*	Environmental DNA	STA ATG GTC TGG CTT AGA CG GCG GCC ATC CAT CTG TAT GT	635	Francis et al. (2005)	95ºC - 5 min, 40 cycles, 95ºC - 40 s, 56ºC - 30 s e 72ºC - 60 s
	nirK	*876 F 1040 R*	30026	ATYGGCGGVCAYGGCGA GCCTCGATCAGRTTRTGGT T	520	Henry et al. (2004)	95ºC - 10 min, 6 cycles, 95ºC - 15 s, 63ºC - 30 s e 72ºC - 40 s, 95ºC - 30 s, 38 cycles, 58ºC - 30s, 72ºC - 40 s
	nosZ	*nosZ 2F nosZ 2R*	17167	CGC RAC GGC AAS AAG GTS MSS GT CAK RTG CAK SGC RTG GCA GAA	267	Henry et al. (2006)	95 ºC - 15s, 60 ºC - 40s, 72 ºC - 40s

†Reference code of cell catalog of the Leibniz Institute DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH*), used in the construction of the standard curves for qPCR analysis.

‡ The PCR products were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, UK)

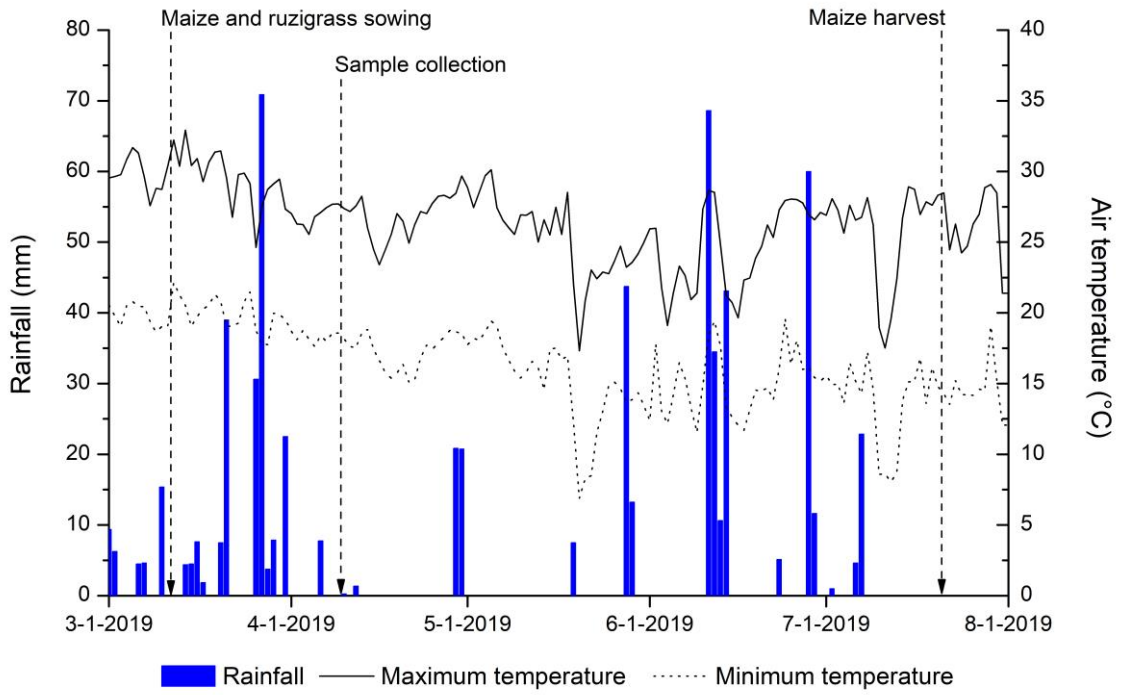


Figure S1. Rainfall and maximum and minimum air temperatures during the experimental period.

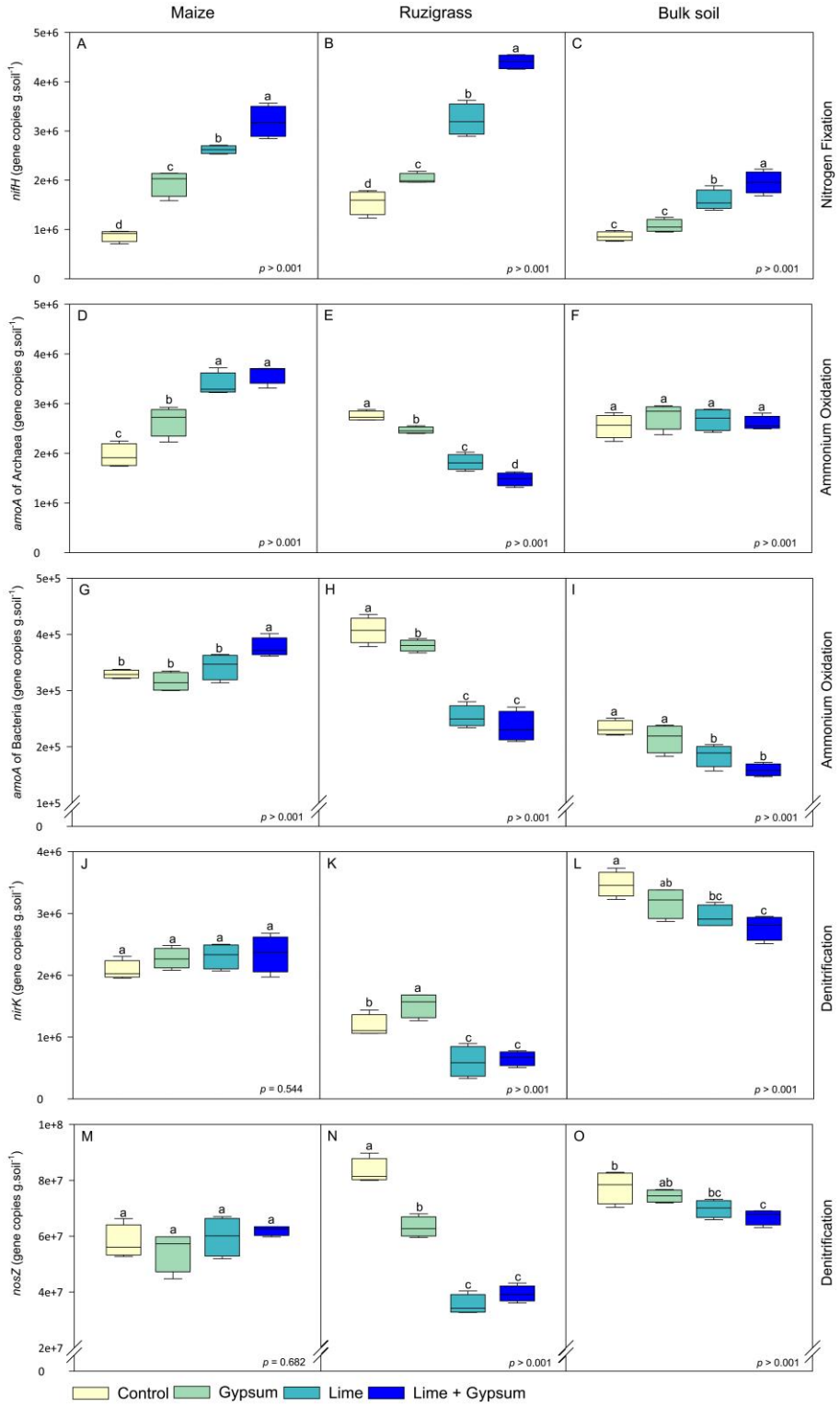


Figure S2. Copy numbers of *nifH*, *amoA* of archaea, *amoA* of bacteria, *nirK* and *nosZ* in rhizospheres of maize (A, D, G, J and M), ruzigrass (B, E, H, K, N) and in bulk soil (C, F, I, L and O) under different treatments (control, gypsum, lime and lime + gypsum). Different lower-case letters indicate significant differences between treatments by LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 12$).

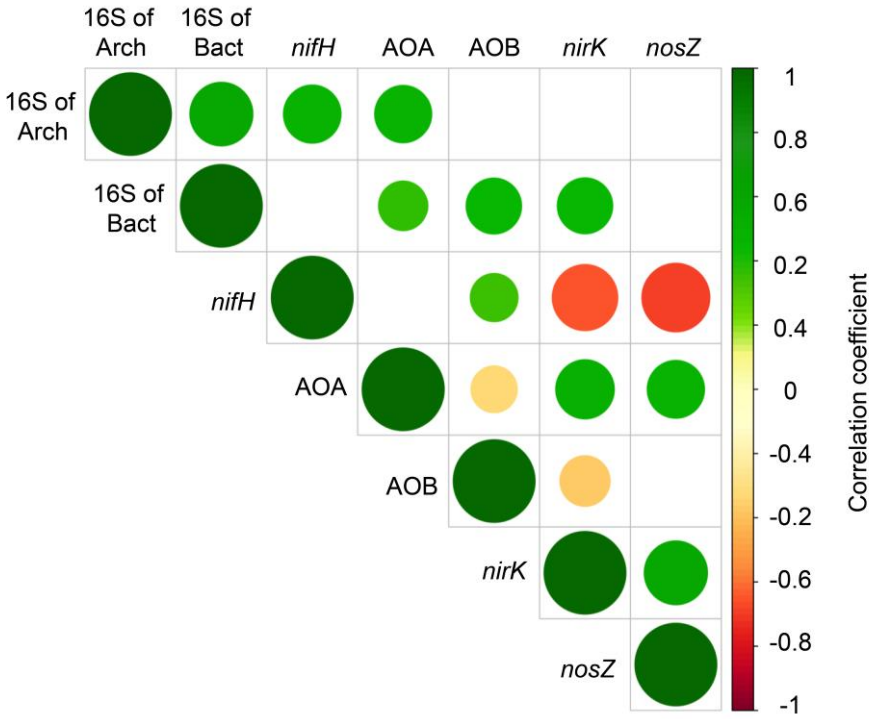


Figure S3. Heatmap of the correlation coefficients (Spearman) between the phylogenetic marker genes (16S rRNA of bacteria and archaea) and total abundance of N-cycle functional genes. Only significant correlations at $p \leq 0.05$ are shown.

CHAPTER 5

CO–APPLICATION OF LIME AND PHOSPHOGYPSUM INCREASES ^{15}N RECOVERY AND REDUCES ITS LOSSES BY MODULATING SOIL FERTILITY, CROP GROWTH AND N CYCLE GENES[5]

Paper in the rules of the *Journal of Cleaner Production*
(ISSN 1161-0301)

Co–application of lime and phosphogypsum increases ^{15}N recovery and reduces its losses by modulating soil fertility, crop growth and N–cycle genes

João William Bossolani[a]; Carlos Alexandre Costa Crusciol[a*]

[a] São Paulo State University (UNESP), College of Agricultural Sciences (FCA), 18610–034, Botucatu, São Paulo, Brazil

[*]Corresponding author:
C. A. C. Crusciol, Email: carlos.crusciol@unesp.br

Abstract

The recovery of nitrogen (N) fertilizers in the soil–plant system can be affected by soil fertility and biological changes caused by the surface application of lime (L) and phosphogypsum (PG) in no–tillage rotation systems. We aimed to assess the role of surface–applied L and/or PG on the fate of ^{15}N–labeled fertilizer, chemical properties, microbial genes (16S rRNA of prokaryotes, *AmoA*, *AmoB*, *nirK*, and *nosZ*) and grain yield of maize [*Zea mays* L.; intercropped with ruzigrass (*Urochloa ruziziensis* cv. Comum]–soybean [*Glycine max* (L.) Merrill] rotation during two growing seasons. Our results demonstrate that L alone and, especially combined with PG (LPG treatment) improved soil fertility resulting in increasing crop grain yield. Moreover, the recovery of ^{15}N–labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$] increased in maize and ruzigrass dry matter, but decreased in soybean grown on the residue of the first growing season. The highest losses of ^{15}N–fertilizer occurred in the control and PG treatments. A large amount of ^{15}N–fertilizer was found in deep layers of PG–amended soil, indicating leaching of fertilizer–derived ^{15}N. Conversely, soil microbial genes revealed that the abundances of denitrifier genes (*nirK* and *nosZ*) increased in the control (no correctives applied), suggesting an increase in denitrification rates caused by the N–fertilizer remaining in the soil. The combination of L with PG is a clear and feasible strategy to increase soil fertility, ^{15}N recovery from fertilizer, and grain yield by crops with consequent much lower environmental pollution by the consequences of nitrification and denitrification processes.

[5] Chapter written in accordance with the rules of Journal of Cleaner Production

Keywords: Soil amendments; Crop rotation; ^{15}N–labeled; Nitrogen losses, Microbial functional genes; Nitrification; Denitrification.

5.1 INTRODUCTION

Agricultural production in tropical regions is constantly limited by soil acidity (Holland et al., 2018; Sumner and Noble, 2003). Acidification is generated by natural processes (*e.g.*, weathering of source material and nutrient cycling), and anthropogenic activities, including agricultural mismanagement practices (Fageria and Nascente, 2014). Low soil pH causes a chain of negative effects on soil biogeochemistry (Holland et al., 2018). These detrimental effects can impair root growth and water and nutrient uptake, leading to yield drawbacks (Bossolani et al., 2021a; Meng et al., 2019). To overcome this issue, the application of lime has been carried out for hundreds of years (Li et al., 2019). Liming is currently used to increase soil pH and base saturation, contributing to increase nutrient availability and complexing exchangeable aluminum (Al^{3+}), which is toxic to plants (Caires et al., 2006; Crusciol et al., 2019).

Until a few years ago, liming was essentially incorporated through plowing and harrowing under conventional till system (Santos et al., 2018). The absence of lime incorporation reduces the effectiveness in the acidity correction, restricting its action to a small soil layer around the lime particles (Soratto and Crusciol, 2008). With the advent of the no–till systems, lime has been applied on the soil surface without incorporation, what conditions limit the reaction of lime to topsoil (Bossolani et al., 2022; Costa et al., 2018). Subsoil effects by liming are frequently reported but depend on soil texture, lime rate (Tiritan et al., 2016), time after application (Carmeis Filho et al., 2017a), and the management of the no–till system (Soratto and Crusciol, 2008). To increase the efficiency of surface liming, studies have emerged combining lime with phosphogypsum (Bossolani et al., 2022, 2021; Soratto and Crusciol, 2008). Phosphogypsum is the residue of the phosphoric acid industry and is basically composed of calcium sulfate ($CaSO_4 \cdot 2H_2O$), about 200 times more soluble than lime (Zoca and Penn, 2017). The dissociation of phosphogypsum in the soil releases calcium (Ca^{2+}) and sulfate (SO_4^{2-}–S) (Caires and Guimarães, 2018). Sulfate forms ligands with Al^{3+}, making it unavailable and allowing root growth to deep layers. In addition, root decomposition creates biopores that increase lime mobility throughout

the soil profile (Tiritan et al., 2016). For this reason, PG acts as an important complement to lime (Bossolani et al., 2021a).

In addition to improving soil chemical properties, several studies have reported a strong influence of lime and PG on soil physics (Carmeis Filho et al., 2017b) and biological properties (Bossolani et al., 2021b, 2020). On the other hand, there are scarce studies that have assessed the interaction between soil amendments with nitrogen (N) fertilizers (Crusciol et al., 2022). Lime and PG can also alter the efficiency of N–fertilizers in agricultural fields since soil amendments application affects microbially–mediated N reactions and N dynamics in the soil–plant system (Holland et al., 2018; Shi et al., 2017). The rise in soil pH following liming also has cascading effects on soil N transformation processes (J. Liu et al., 2018). N–fertilizers are one of the most expensive inputs for crop production and when used improperly (*i.e.*, under– or overfertilization), limit the maximum performance of crops and/or pollute the environment (Ashitha et al., 2021; Fageria and Nascente, 2014).

The reaction of ammonium–based fertilizers can lead to soil acidification as a result of nitrification (Yang et al., 2021). Therefore, the application of lime and PG in association with ammonium–based fertilizers can neutralize the acidifying effect, stimulate nitrification in acidic soils and improve N–fertilizer uptake by crops (Garbuio et al., 2011). To mitigate N losses from the system (e.g., leaching, volatilization, N_2O emission, etc), N–recovery by plants should be increased as much as possible. Therefore, the application of lime and PG is a way to enhance the root growth to deeper horizons where fertilizer–derived N can be vertically displaced (Weligama et al., 2010). After harvest, crop residues become a temporary N pool that can be accessed by crops grown in sequence through their decomposition and mineralization, decreasing N losses from fertilizer (Yang et al., 2021).

Research related to understanding the dynamics of N in tropical agricultural systems, especially based on crop rotation between grasses and legumes is incipient. Moreover, the recovery of residual N–fertilizer [applied to maize (*Zea mays* L.) intercropped with forage grass] by soybean [*Glycine max* (L.) Merrill] remains unclear, especially when soil amendments are associated with N–fertilizers. Here we hypothesized that crops (maize + ruzigrass, and soybean in rotation) established in soils broadcasted with lime and PG recover a greater amount of ^{15}N–fertilizer than when established in acidic soils, which results in less N loss to the environment. To test this hypothesis, we examined the effects of the surface application of lime (L),

combined or not with phosphogypsum (PG) on maize (intercropped with ruzigrass)–soybean rotation on (i) soil fertility (ii) biomass and grain yield by the crops (iii) ^{15}N recovery in the soil–plant system, and (iv) abundance of N–cycle genes.

5.2 MATERIAL AND METHODS

5.2.1 Site description

A long–term (18 years) field experiment [registered by the Global Long Term Agricultural Experiments Network (GLTEN), Rothamsted Research, UK; https://www.glten.org/experiments/62] set in Botucatu, São Paulo State, Brazil (22°83′3″ S, 4°42′64″ W, 765 m a.s.l.) was used in this study. This experiment is based on surface applications of lime (L) and/or phosphogypsum (PG) in an agricultural system with crop rotation under no–till. According to the Köppen–Geiger's climate classification system, the climate in this region is classified as mesothermic type (Cwa), with a humid subtropical zone, presenting dry winters and hot summers. The average (1,956–2,020) annual precipitation is ~1,360 mm, whereas the mean annual air temperature ranged from 15.3 to 26.1 °C. The climatic conditions during the experimental period are in Fig. 1A. The soil from the experimental area is a sandy clay loam kaolinitic thermic Typic Haplorthox (particle size composition: 347 g kg^{-1} clay, 108 g kg^{-1} silt, and 545 g kg^{-1} sand; Soil Survey Staff, 2014). Prior to establishment of the study (2002), initial soil properties were determined at 0–20 cm depth according to van Raij et al. (2001): soil pH ($CaCl_2$) 4.2; soil organic C (SOC) 12.2 g kg^{-1}; P_{resin} 9.2 mg kg^{-1}; exchangeable K 1.2 $mmol_c$ kg^{-1}; exchangeable Ca^{2+} 14 $mmol_c$ kg^{-1}; exchangeable magnesium Mg 5 $mmol_c$ kg^{-1}; total acidity at pH 7.0 (H+Al) 37 $mmol_c$ kg^{-1}, cation exchange capacity (CEC) 57 $mmol_c$ kg^{-1}; base saturation (BS) 35%; and Al saturation (AS) 65%. In addition, the clay content at the 20–40 cm soil depth was determined (360 g kg^{-1}) for PG rate calculation.

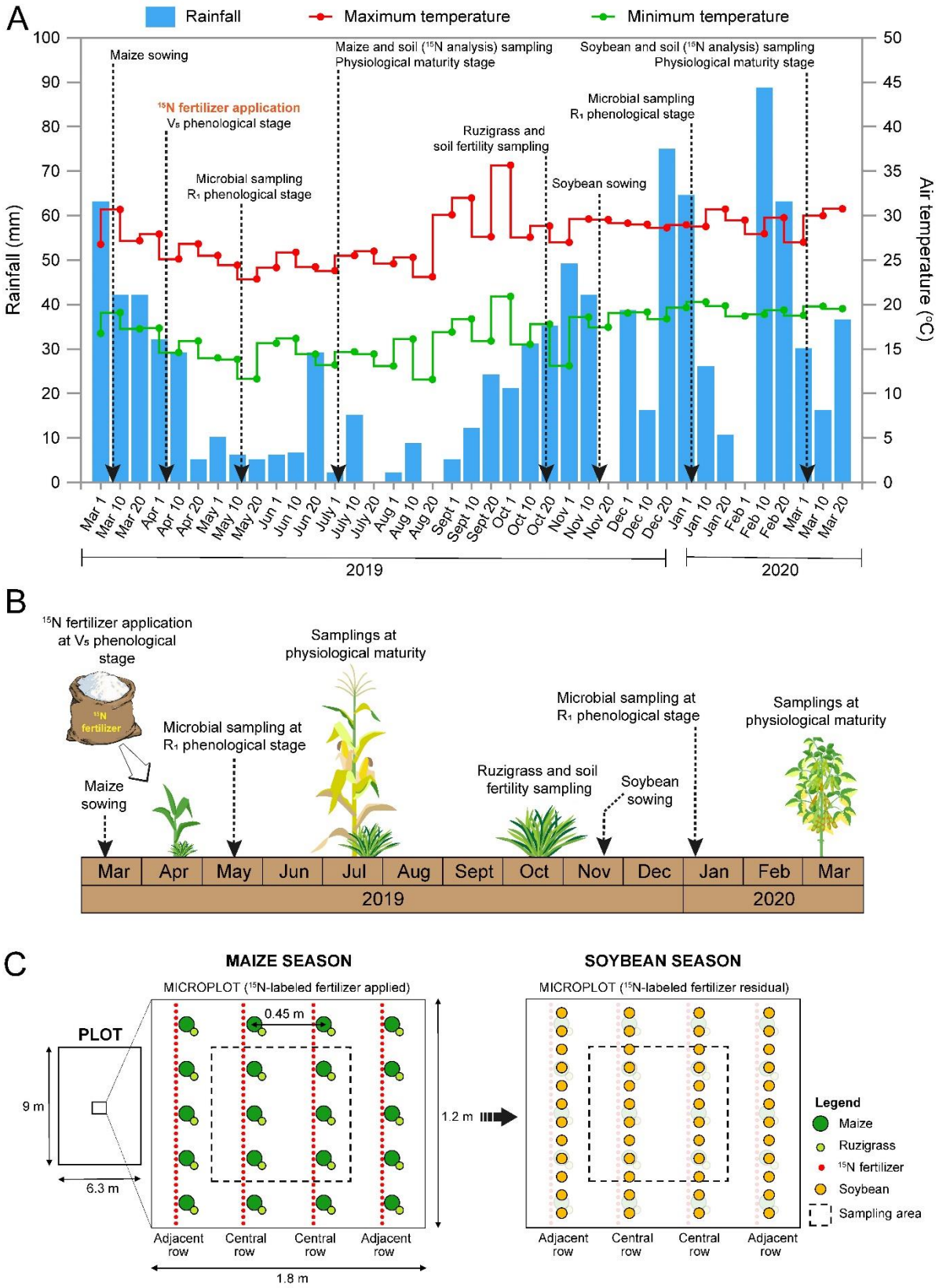


Figure 1. Weather conditions (A), main activities during experimental period (A and B), and schematic representation of the plot, ^{15}N–labeled fertilizer microplot, in addition to the ^{15}N–fertilizer distribution (~3 cm from the maize row), sampling area, and sowing of soybean on the residues of maize + ruzigrass (C).

5.2.2 Experimental design and field management

Four different treatments with four replicates each were tested in a randomized complete block design. The plot sizes were 57 m^2 (9.0 × 6.3 m). Treatments were (*i*) control (no soil amendments applied); (*ii*) exclusive application of PG; (*iii*) exclusive application of L; and (*iv*) combined application of L and PG (LPG).

Over the 18–year experimental period, the field area received four reapplications of treatments (2002, 2004, 2010, and 2016). Reapplications took place based on the results of base saturation performed annually. The dolomitic L dose was defined following the recommendations of van Raij et al. (1997) for fertilization and liming for the state of São Paulo, Brazil, which considers BS and CEC values. From 2002 to 2010, the calculation was based on the results obtained in the 0–20 cm soil depth layer. Conversely, the calculation for the last reapplication considered the soil layer of 0–40 cm, and the result was multiplied by a factor 2 to obtain de doible of the reccomended dose. This change was adopted due to the findings of Carmeis Filho et al. (2017a), where the authors, studying this same field experiment, found that the L dose considering the 0–20 cm layer underestimates the ideal dose of L for intensive tropical agricultural systems. The revised calculation resulted in 13 Mg ha^{-1} of L to increase the BS to 70%. When liming occurred, PG was also reapplied. In the first three applications of PG, the dose calculation was based on the recommendations of van Raij et al. (1997) by multiplying the clay content in the 20–40 cm layer by a factor of 6, resulting in a dose of 2.1 Mg ha^{-1}. Recently, Caires and Guimarães (2018) proposed a new method for recommending PG based on increasing the Ca^{2+} saturation in the effective CEC (ECEC) to 60% in the 20–40 m soil layer. The new calculation resulted in the application of 10 Mg ha^{-1} of PG in 2016.

This study characterizes the residual effects of L and PG application in the third year after the last soil amendment reapplication (2016). The cropping history from 2002 to 2020, as well as the details of previous treatment applications, are given in Table S1.

5.2.3 Crop management

The chronological details of the events are shown in Fig. 1AB. Maize (hybrid P3707VYH, DuPont Pioneer, Rio Grande do Sul, Brazil) was planted in March 2019 at a row spacing of 0.45 m to achieve a final stand of 65,000 plants ha^{-1}. The grain crop was intercropped (same row) with ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (Syn. *Brachiaria ruziziensis* Germ. & Evrard)], at a seed density of 10 kg ha^{-1}. Each plot received 98 kg ha^{-1} P_2O_5 and 56 kg ha^{-1} K_2O at planting time. Nitrogen application was split twice, with 28 kg ha^{-1} N at planting and 100 kg ha^{-1} N topdressed at phenological stage V_5 (five leaves with visible leaf collars, Ritchie et al., 1993). Topdressed N–fertilizer was hand–applied to the soil surface in single–side banding at ~2.5 cm from the maize row. The crop was harvested in July 2019, and the maize stover (stalk, leaves, sheaths, tassel, core cob, and straw cob) was left in the field. After maize harvest, ruzigrass remained growing until October 2019, when it was chemically terminated using glyphosate (2.5 kg ha^{-1} a.i.). In November 2019, soybean (cultivar TMG 7062 IPRO, TMG – Tropical Melhoramento & Genética, Paraná, Brazil) was sown over the ruzigrass residue. At planting time, fertilization occurred with 60 kg ha^{-1} P_2O_5, and 60 kg ha^{-1} K_2O. The seeds were treated with fungicides (carboxin + thiram up to 100 g + 100 g a.i. 100 kg of $seeds^{-1}$) prior to inoculation and planting. Soybean harvest occurred in March 2020. Phytosanitary treatments occurred as necessary and recommended for maize and soybean.

5.2.4 ^{15}N microplots establishment

Unconfined microplots (1.8 × 1.2 m) were set up in each treatment during the maize + ruzigrass season (Fig. 1C). All agricultural management in the microplots matched those of the main plots. Each microplot consisted of four rows of maize, with five plants per row (~4.2 plants m^{-1}). ^{15}N–labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$] with an abundance of 6.31 atom % ^{15}N excess was obtained from Sigma–Aldrich Inc. (St. Louis, MO, USA). ^{15}N–labeled fertilizer application occurred in topdressing (100 kg ha^{-1} N) at the V_5 maize stage. The microplots received only ^{15}N–labeled fertilizer, whereas the main plot received unlabeled ammonium sulfate. Maize stover returned to the microplot after lab processing to ensure the cycling of the ^{15}N present in the plant

residues. After maize harvesting, the microplot remained identified to assess the recovery of residual ^{15}N–labeled fertilizer by soybean.

5.2.5 Sampling procedure of ^{15}N–labeled material and isotopic analyses

At maize physiological maturity [R_6 stage; (Ritchie et al., 1993)], six maize plants were clipped to ground level in the middle of each microplot. Maize plants were partitioned into vegetative fractions (stalk, leaves, sheaths, tassel, core cob, and straw cob), and grains. All vegetative fractions from each microplot were mixed (herein defined as stover), chopped with a forage grinder, the fresh samples were oven–dried at 65 °C to constant weight to obtain the dry weight. The same was done for the grain fraction. The subsample from dried stover and grains was ground in a Wiley mill (0.50–mm sieve). The remaining stover was returned to the microplots.

The ruzigrass biomass was sampled in October 2019 before its chemical desiccation. In each microplot, an area of 0.25 m^2 was collected (at ground level). Ruzigrass biomass samples were oven–dried. A subsample from dry plant material was also ground in a Wiley mill. Similar to maize, the remaining biomass returned to the microplots. With regards to soybean, at the beginning of physiological maturity [R_7 phenological stage; (Fehr and Caviness, 1977); March 2020], before leaf senescence, six plants were sampled and separated into vegetative fractions [stem, leaves (including petiole), and pods], and grains. All vegetative fractions were pooled and termed as soybean stover. The same drying and grinding procedures were carried out. Plants from each main plot (maize stover and grains, ruzigrass, and soybean stover and grains) were also subjected to the same procedure above to assess the natural ^{15}N abundance. All milled plant tissue was used to determine total N concentration and ^{15}N measurements.

Soil sampling occurred using a core sampler at seven depths (0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm). Six soil samples were collected per microplot and combined into one sample per depth per microplot. Three sample points were collected on the maize rows, which received ^{15}N–labeled fertilizer and three other points were collected between the maize rows. Soil samples were oven–dried and ground in a ball mill and passed through a 100 mesh sieve (0.15–mm sieve). Total N concentration and ^{15}N measurements occurred in these soil samples. The natural ^{15}N abundance in the soil was also measured. To estimate the soil N accumulation for each

soil depth and treatment, the soil bulk density was assessed through the volumetric ring method (Blake and Hartge, 1986), which was performed during the ruzigrass season. All plant tissue (maize, ruzigrass, and soybean), as well as soil samples, were analyzed for total N concentration and ^{15}N abundance using an automatic elemental analyzer (Flash EA, Thermo Scientific, Germany) interfaced with an isotope ratio mass spectrometer (CF–IRMS, Delta V, Thermo Scientific, Germany).

5.2.6 ^{15}N calculations

A range of variables was calculated to determine the ^{15}N recovery in the soil–plant system, including the amount of N derived from fertilizer (Ndff), ^{15}N recovery by crops, soil ^{15}N retention in each soil layer and across all soil depths (down to 100 cm), and the unrecovered ^{15}N. From here, we considered the first season as maize + ruzigrass, while the second season refers to soybean. The ^{15}N recovery was determined according to the following equations:

$$Ndff\ (kgha^{-1}) = (a/b\ ) \times TN \quad \text{(Eq. 1)}$$

$$^{15}N\ recovery\ (\%) = (Ndff/NFR)\ \times 100 \quad \text{(Eq. 2)}$$

$$^{15}N\ \text{unrecovered}_{FS}\ (\%) = 100 - {}^{15}N\ recovery_{TFS} \quad \text{(Eq. 3)}$$

$$^{15}N\ \text{remaining}\ (\%) = {}^{15}N\ recovery_{TFS} - {}^{15}N\ recovery_{maize\ grain} \quad \text{(Eq. 4)}$$

$$^{15}N\ \text{unrecovered}_{SS}\ (\%) = {}^{15}N\ remaining - {}^{15}N\ recovery_{SS} \quad \text{(Eq. 5)}$$

where *Ndff* is the N derived from fertilizer; *a* and *b* are the ^{15}N enrichment (atom % ^{15}N excess) in the plant (maize/ruzigrass/soybean) fractions (stover and grain) or soil, and substrate (fertilizer), respectively (the natural abundance of 0.368 atom % ^{15}N was considered in the calculations); *TN* represents the N accumulation (kg ha^{-1}) in the plant fractions or soil; *^{15}N recovery* is the percentage of fertilizer N recovery; *NFR* is the N–fertilizer rate applied (kg ha^{-1}); *^{15}N unrecovered$_{FS}$* and ^{15}N *unrecovered$_{SS}$* are the percentage of N–fertilizer unaccounted (*i.e.*, potential losses) after maize + ruzigrass (^{15}N–labeled fertilizer applied in the maize + ruzigrass season), and after soybean s(^{15}N–labeled fertilizer residual in the second season); *^{15}N recovery$_{TFS}$* is the total N recovery (%; sum of maize stover and grain, ruzigrass, and soil) in the maize + ruzigrass season; *^{15}N remaining* is the amount of ^{15}N available in the system before soybean grown; *^{15}N recovery$_{maize\ grain}$* is the amount of ^{15}N exported with maize grains; *^{15}N recovery$_{TSS}$* the total N recovery (%; sum of soybean stover and grain and soil) in

the second season.

5.2.7 Soil sampling and chemical analyses

To measure soil fertility status, composite soil samples (*n* = 8) were taken from 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm layers from each plot using a soil probe. The sampling procedure was carried out in October of 2019, before ruzigrass desiccation (3 years after the last application of L and PG, and 17 years after the onset of the experiment) The soil was air–dried and ground to pass through a 2–mm sieve for chemical analysis.

Soil pH was determined in a 0.01 M $CaCl_2$ suspension [2:5 soil:solution, v:v; (van Raij et al., 2001)]. Soil organic C was determined according to Heanes (1984). Exchangeable Al^{3+} was extracted using 1 M KCl and determined by titration with 0.025 M NaOH solution. Exchangeable Ca^{2+} and Mg^{2+} were extracted using ion exchange resin and determined using atomic absorption spectrometry (van Raij et al., 2001). SO_4^{2-}–S was extracted by 0.01 M calcium phosphate solution (Bardsley and Lancaster, 1960), and determined by turbidimetry.

5.2.8 Soil DNA sampling, extraction and qPCR analyses

Soil sampling for microbiological analysis occurred after the maize and soybean harvest at the 0–10 cm layer. Bulk soil samples (*i.e.*, six individual samples to form a composite sample) were taken between rows at the R_1 stage of maize and soybean. Total DNA was extracted from 0.25 g of soil using the DNeasy Power Lyzer Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Soil DNA quality and concentration were measured using NanoDrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, USA) and 1% sodium boric acid agarose gel electrophoresis (Brody and Kern, 2004). Extracted DNA samples were stored at −20 °C until further analysis.

The abundance of total bacteria (*16S rRNA* gene of bacteria) and archaea (*16S rRNA* gene of archaea), nitrifiers (*AmoB*–ammonia monooxygenase gene of bacteria, and *AmoA*–ammonia monooxygenase gene of archaea), and denitrifiers (*nirK*–nitrite reductase gene and *nosZ*–nitrous oxide reductase gene) was assessed by qPCR

technique using the StepOnePlus Real–Time PCR System with 96–well plates (Applied Biosystems, Foster City, CA). The standard curves were generated from a 10–fold serial dilution (10^8 to 10^3) of a known amount of target gene, which was previously amplified by PCR. Details of primers and the respective reaction conditions for gene amplifications are described in Table S2. All analyzes were performed in triplicate. The analyzes of melting curves were performed from 68 to 95 °C, and all standard curves had R^2 greater than 0.98. The results were analyzed using the StepOnePlus™ Real–Time software version 2.2.2 (Applied Biosystems, Foster City, CA, USA). Gene abundance from each sample were transformed into the number DNA copies g fresh soil^{-1}.

5.2.9 Statistical analysis

All statistical analyses were performed using R (version 4.1.3, The R Foundation for Statistical Computing, Boston, MA, USA). Data were tested for normality [Anderson–Darling test; (Nelson, 1998)] and homoscedasticity [Levene's test; (Levene, 1960)]. When significant, means were separated using the modified t–test [Fisher's protected least significant difference (LSD), at $p \leq 0.05$]. Redundancy analysis (RDA) was performed to determine the correlations among soil fertility × ^{15}N recovery, soil fertility × N cycle genes, and for N cycle genes × ^{15}N recovery of maize and soybean, separately. RDA triplots were generated using Canoco (version 4.5, Biometrics, Wageningen, Netherlands). Monte Carlo permutation test was applied with 999 random permutations to verify the significance of soil fertility, ^{15}N recovery, and N–cycle genes response. One–way PERMANOVA analyses (Anderson, 2005) were performed using R (version 4.1.3, The R Foundation for Statistical Computing, Boston, MA, USA), and was used to group treatments by similarity.

5.3 RESULTS

5.3.1 Soil profile fertility

Contrasting levels of soil fertility occurred after three years of the last soil amendments reapplication (Table 1). Soil acidity reduced across the entire soil profile when L was applied (regardless of PG combination) compared with control and PG.

As expected, PG was not efficient in altering the soil pH compared with control. Soil organic C content was higher in LPG than L alone up to 20 cm depth. The SOC contents in PG–amended soil was higher than the control up to 20 cm depth. Ca–based soil amendments (PG, L, and LPG) increased Ca^{2+} concentration in all depths compared with control, and had the following trend: LPG > L > PG. Liming treatments also increased Mg^{2+} concentration throughout the soil profile, however, in layers below 40 cm, LPG presented the highest values compared with L alone. Lime and LPG treatments were equally efficient in reducing the Al^{3+} concentration in the soil profile, especially in top–layers (*e.g.*, down to 40 cm). Application of PG also reduced Al^{3+} concentration compared with control, but at lower levels than L and LPG. The most notable effect of PG occurred by increasing concentrations of SO_4^{2-}–S, while LPG had the highest concentrations of SO_4^{2-}–S across the soil profile. Lime and PG treatments did not differ in SO_4^{2-}–S concentrations up to 10 cm, but for subjacent layers, PG had the highest values.

Table 1. Soil fertility in response to soil amendments [control (no soil amendments applied), phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] at seven stratified soil layers up to 100 cm depth.

Soil depth (cm)	SA‡	pH ($CaCl_2$)	SOC (g kg^{-1})	Ca^{2+}	Mg^{2+}	Al^{3+}	SO_4^{2-}–S
				——— ($mmol_c$ kg^{-1}) ———			(mg kg^{-1})
0 – 5	Control	4.08 b†	15.9 c	11.0 d	8.61 b	4.45 a	9.4 c
	PG	4.32 b	17.1 b	45.0 c	10.4 b	2.32 b	23.6 b
	L	6.00 a	17.8 ab	61.7 b	25.7 a	0.16 c	21.2 b
	LPG	6.08 a	18.3 a	78.5 a	26.6 a	0.06 c	32.4 a
5 – 10	Control	3.68 b	14.3 c	9.90 d	7.75 b	6.68 a	8.45 c
	PG	3.89 b	15.4 b	40.5 c	9.35 b	3.47 b	21.3 b
	L	5.40 a	16.0 ab	55.5 b	23.1 a	0.24 c	19.0 b
	LPG	5.48 a	16.5 a	70.6 a	23.9 a	0.08 c	29.2 a
10 – 20	Control	4.01 b	11.9 d	9.11 d	5.00 b	14.9 a	12.1 d
	PG	4.11 b	13.1 c	19.0 c	6.04 b	13.0 b	32.6 b
	L	5.01 a	14.4 b	35.0 b	16.2 a	1.06 c	25.6 c
	LPG	5.17 a	15.3 a	44.6 a	16.7 a	1.08 c	42.5 a
20 – 40	Control	3.98 b	10.8 b	8.81 d	2.77 b	15.5 a	15.6 c
	PG	4.03 b	11.2 b	13.6 c	4.29 b	12.9 a	40.8 b
	L	4.53 a	13.0 a	30.2 b	12.7 a	2.85 b	31.2 c
	LPG	4.62 a	14.0 a	38.3 a	12.6 a	2.95 b	59.4 a
40 – 60	Control	3.83 b	10.2 c	5.71 d	2.84 c	16.7 a	16.3 c
	PG	3.87 b	11.1 bc	8.38 c	3.47 c	15.4 a	43.6 b
	L	4.14 a	11.9 ab	17.5 b	11.1 b	9.40 b	37.3 c
	LPG	4.27 a	12.5 a	22.2 a	13.0 a	7.58 b	69.8 a
60 – 80	Control	3.76 b	9.22 c	1.92 c	2.30 c	20.5 a	21.8 d
	PG	3.88 b	10.1 bc	5.61 b	2.03 c	19.0 a	54.1 b
	L	4.13 a	10.6 ab	11.9 a	10.1 b	11.3 b	43.5 c
	LPG	4.16 a	11.5 a	12.2 a	14.7 a	9.09 b	72.6 a
80 – 100	Control	3.75 b	8.60 b	1.42 d	1.35 c	20.9 a	23.7 d
	PG	3.88 b	8.77 b	3.83 c	1.18 c	19.4 a	64.4 b
	L	4.06 a	9.31 ab	7.21 b	2.34 b	11.7 b	31.7 c
	LPG	4.05 a	9.92 a	9.17 a	4.07 a	9.45 b	72.8 a

† Different lowercase letters for each soil layer indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$; ‡ Soil amendments (SA).

5.3.2 Aboveground dry matter yield

In general, in the first growing season, maize plants had, on average, 71% higher aboveground (shoots) dry matter (stover + grain) production in limed treatments (L and LPG) compared with control and PG (Fig. 2A). Ruzigrass biomass followed the

results observed for maize. In the second growing season, soybean aboveground dry matter production in LPG treatment was, on average, 16%, 70%, and 108% higher than L, PG, and control, respectively (Fig. 2B).

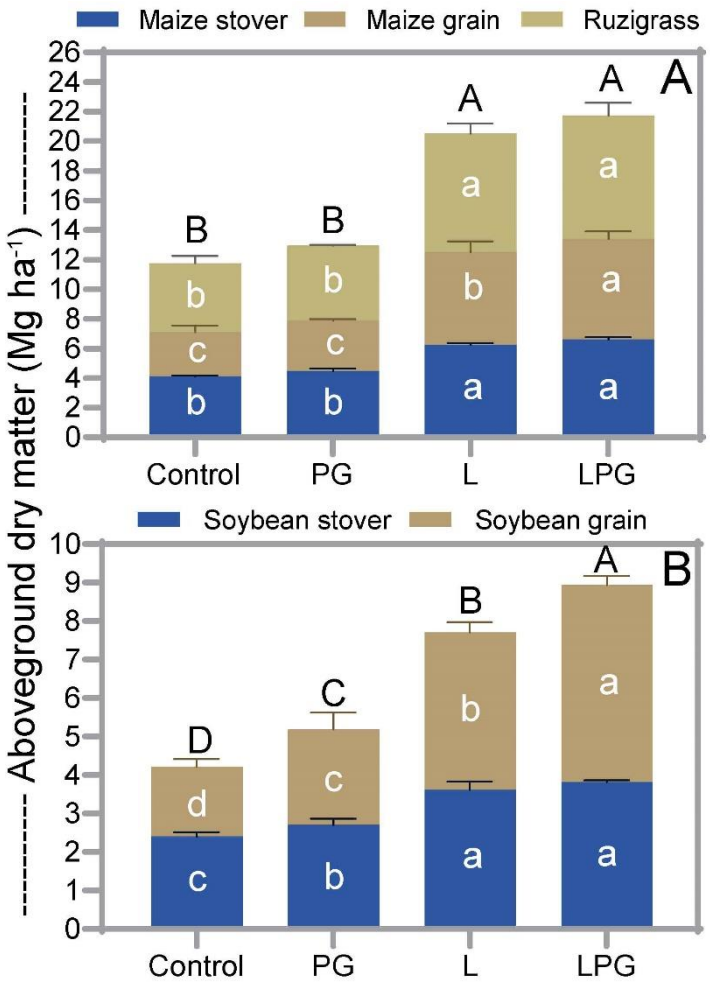


Figure 2. Aboveground (stover + grain) dry matter yield in the first (maize and ruzigrass) (A), and second (B; soybean) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase or capital letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 4).

5.3.3 ^{15}N Recovery in soil–plant system

^{15}N recovery by maize fractions (stover and grain) and ruzigrass was higher in L, and especially, LPG–amended soil (Fig. 3A).

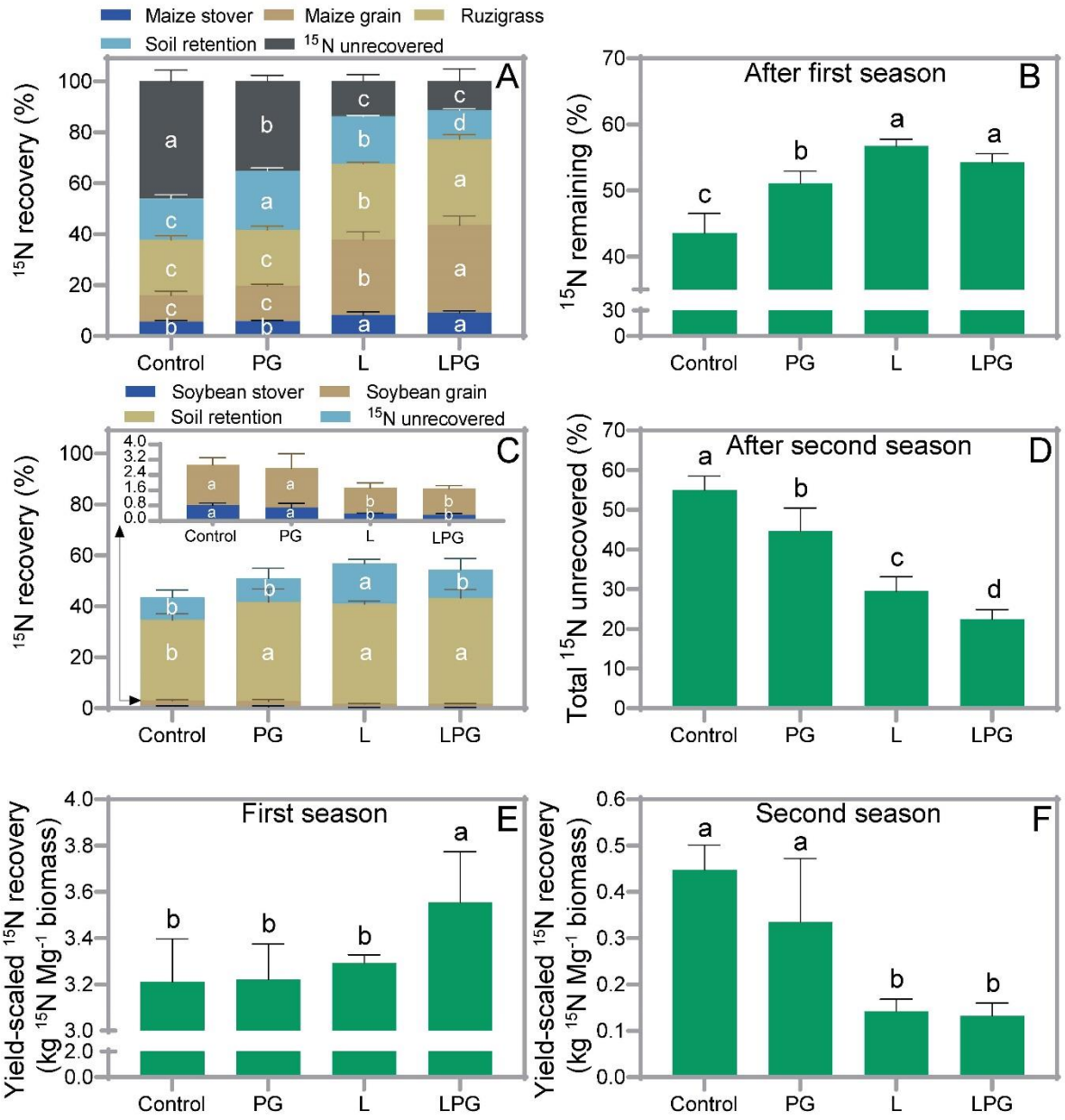


Figure 3. ^{15}N recovery in each compartment (plant, soil or unrecovered) from the first (A), and second (C) growing seasons; ^{15}N–fertilizer remaining after the first growing season (B), total ^{15}N unrecovered (first + second growing seasons; D); and yield–scaled ^{15}N recovery related to the total biomass production in the first (E), and second (F) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 4).

There was no difference between L and LPG, resulting in values ~48% higher than the control and PG. On the other hand, ^{15}N recovery of maize grain and ruzigrass biomass had the highest values on LPG treatment. In general, ~75% of the ^{15}N–fertilizer found in maize shoots was exported by grains. LPG treatment increased by

232%, 150%, and 16% the ^{15}N recovery by grains compared with control, PG, and L, respectively. Ruzigrass had a similar trend: LPG–amended soil increased ^{15}N recovery by 55%, 37%, and 13% compared with control, PG, and L, respectively. Soil retention (down to 100 cm) of ^{15}N–fertilizer was higher in PG (23 kg ha^{-1}), followed by L (19 kg ha^{-1}), control (16 kg ha^{-1}), and LPG (11 kg ha^{-1}). Unrecovered ^{15}N had an inverse pattern to ^{15}N recovery by maize and ruzigrass plants, where the highest losses of fertilizer-derived ^{15}N occurred in the control (46%), followed by PG (35%), L (14%) and LPG (11%).

At the end of the maize + ruzigrass season, the amount of ^{15}N found in the soil–plant system was assigned as ^{15}N remaining (*i.e.*, ^{15}N recovered by plants and soil retention, excluding the amount exported by grains; Fig. 3B). Thus, ^{15}N remaining was higher in L and LPG, which on average were ~27% and 9% higher than those of control and PG, respectively. Overall, ~52% of the ^{15}N–fertilizer applied to the maize remained in the soil–plant system before the soybean planting.

At soybean harvest, ^{15}N recovery of stover and grain showed the opposite trend to that obtained by maize (Fig. 3C). Overall, ^{15}N recovery of stover and grain in L and LPG treatment was 63% and 33% lower, respectively, compared with the average of control and PG. In addition, ^{15}N recovery in soybean shoots was on average 92% lower than that of maize. Regards the soil retention after soybean, the control treatment was the lowest (32%) compared with the average of the other treatments (40%). The amount of unrecovered ^{15}N following soybean was also affected by the soil amendments. The highest loss occurred in L–amended soil (16%), whereas the average of other treatments (PG and LPG) reached 10%. At the end of the experiment, the total amount of unrecovered ^{15}N (sum of the first and second growing seasons) had this series: control (55%) > PG (45%) > L (29%) > LPG (22%; Fig. 3D).

Based on the values of total ^{15}N recovery from each season and dry weight production, we calculated the yield–scaled ^{15}N recovery (Fig. 3E and F). In the maize + ruzigrass season, there was greater use of ^{15}N–fertilizer for each Mg ha^{-1} of biomass produced in the LPG treatment (3.5 kg ^{15}N Mg^{-1} biomass) compared with other treatments (average of 3.2 kg ^{15}N Mg^{-1} biomass; Fig. 3E), whereas the opposite occurred in the soybean season (Fig. 3F). The accumulation of fertilizer-derived ^{15}N in biomass production decreased from 0.39 kg ^{15}N Mg^{-1} biomass (average of control and PG) to 0.14 kg ^{15}N Mg^{-1} biomass (average of L and LPG).

5.3.4 ^{15}N retention along the soil profile

After maize and soybean harvest, ^{15}N retention along the soil profile was influenced by treatments (Fig. 4). After maize harvest, the uppermost soil layers (0–20 cm) from L and PG–amended soils retained the greater amount of ^{15}N–fertilizer, whereas in deeper layers (20–100 cm), the highest ^{15}N retention occurred in PG–amended soil (Fig. 4A). After soybean harvest, soils amended with LPG, followed by L, presented the highest ^{15}N retention (Fig. 4B) down to 40 cm layer. Conversely, as observed after maize harvest, in layers below 60 cm depth, the highest ^{15}N retention also occurred in PG–amended soil.

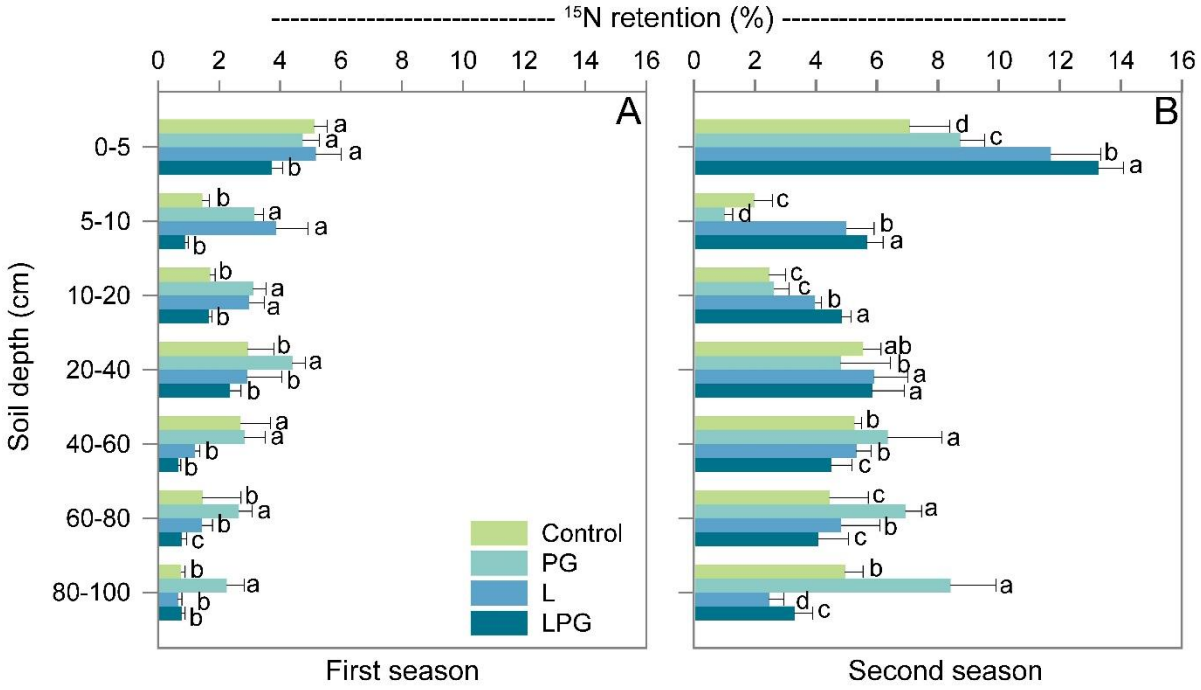


Figure 4. ^{15}N retention at seven stratified soil layers up to 100 cm depth after first (A), and second (B) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters for each soil layer indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

5.3.5 Phylogenetic and functional (N cycle) marker genes

During maize season, total bacteria abundance (16S rRNA gene) from L and LPG treatment increased by ~20% and 25% compared with PG and control, respectively (Fig. 5A). In addition, total bacteria abundance during soybean season

was also highest in LPG–amended soil. On the other hand, total archaea abundance was more sensitive to changes caused by treatments (Fig. 5B). For both crops, L and mainly LPG increased the abundance of total archaea compared with control and PG treatments. Considering the average between L and LPG, total archaea abundance during maize season increased by 81%, and 265% compared with PG and control treatments, respectively, whereas also increased the archaeal abundance during soybean season by 18%, and 58% compared with PG and control, respectively.

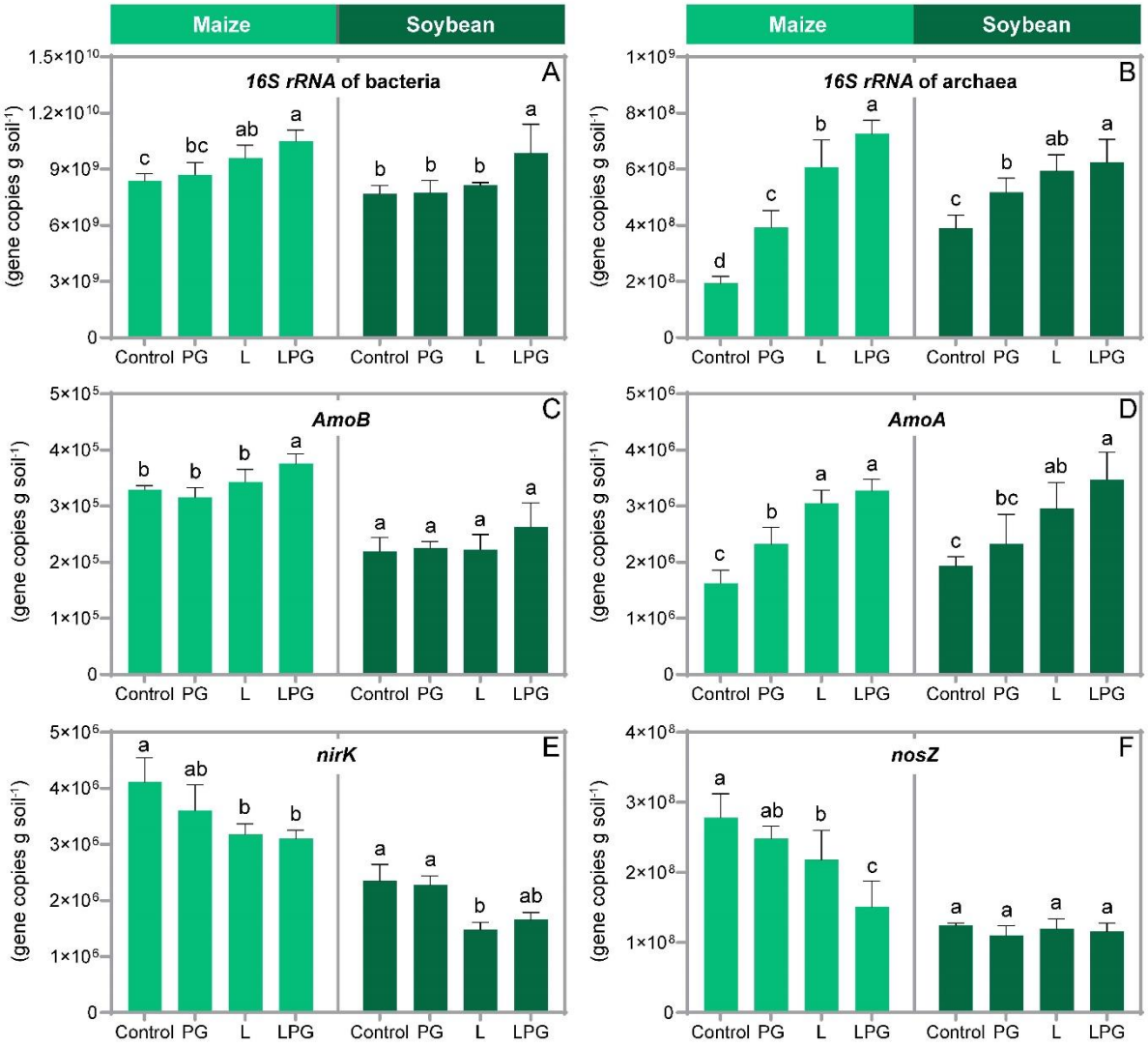


Figure 5. Copy numbers of phylogenetic (16S rRNA) marker genes of bacteria (A) and archaea (B), and functional marker genes related to N cycle as *amoB* (C), *amoA* (D), *nirK* (E) and *nosZ* (F) during maize and soybean seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 12).

To evaluate soil microbial communities related to N cycle, functional marker genes were also quantified. The abundance of *AmoB* (bacteria) gene changed by the treatments only during maize season, where LPG treatment had ~14% higher abundance of this gene compared with the other treatments (Fig. 5C). By contrast, the abundances of *amoA* (archaea) gene in both crop seasons increased similarly as occurred in total archaea. For maize, L and LPG had the highest *AmoA* gene abundances, LPG was higher than PG and control (Fig. 5D).

The copy numbers of *nirK* and *nosZ* genes, both related to the denitrification process, decreased in treatments with L and LPG, especially during maize cultivation (Fig. 5EF), except for *nosZ* during soybean season, which was not altered by the treatments. For both *nirK* and *nosZ* during maize grown, gene abundance was higher in the control in comparison with L and LPG. Lastly, *nirK* during soybean season was also higher for these treatments relative to L.

5.3.6 Linking plant–soil–genes data

Our first RDA considered the relationship between soil fertility and ^{15}N recovery in the soil–plant system for both growing seasons (Fig. 6A). Here, the sum of the two axes (RDA_1 = main variation; RDA_2 = remaining variation) indicated that the soil factors explained 88% of the all variation in the ^{15}N recovery. PERMANOVA analysis segregated the four treatments into distinct groups. Soil pH and Ca^{2+} concentration as the main soil factors responsible for all variation in ^{15}N recovery. The vectors also showed the highest ^{15}N recovery by maize and ruzigrass in the L and LPG–amended soils, whereas the highest ^{15}N recovery by soybean occurred in the control treatment. Control treatment also represented the biggest losses of the ^{15}N–fertilizer in the maize season.

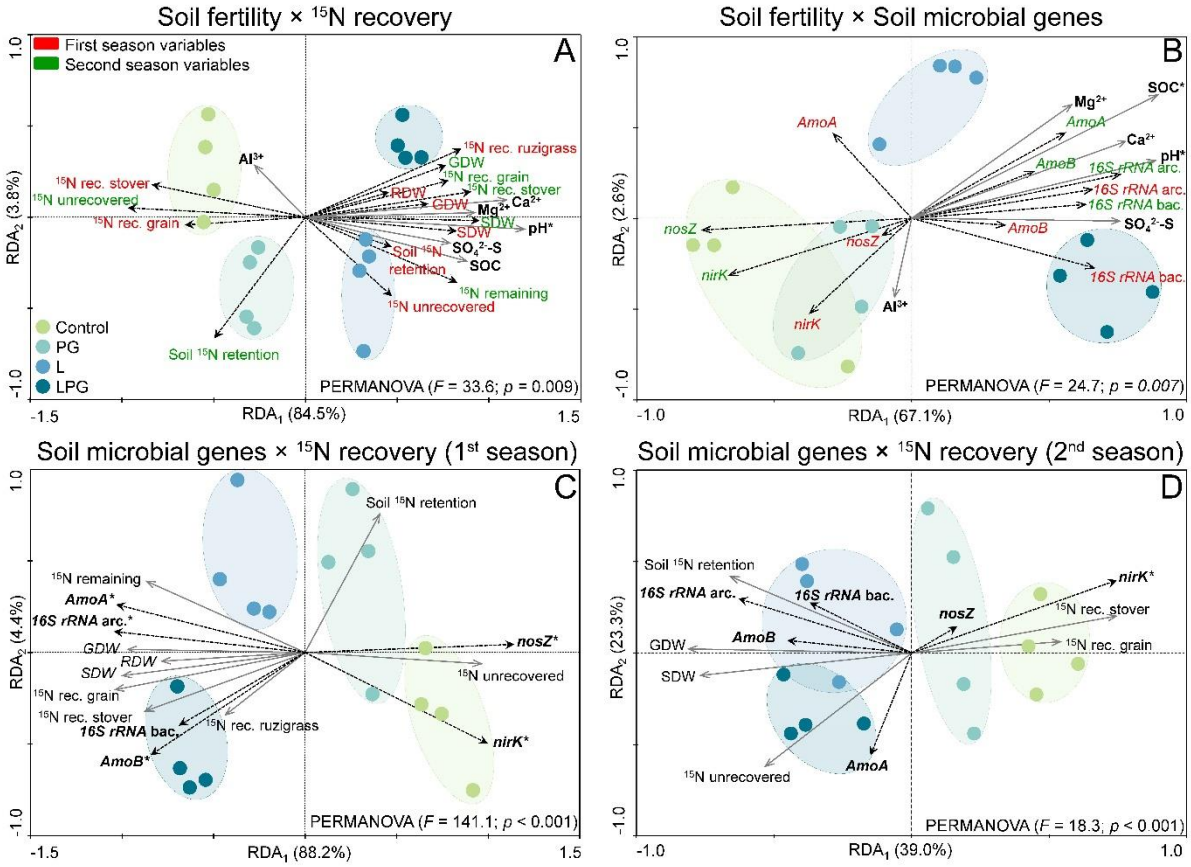


Figure 6. Redundancy analysis (RDA) triplot showing the relationship between the soil fertility × ^{15}N recovery from both first and second growing seasons (A), soil fertility × N microbial genes from both first and second growing seasons (B), soil microbial genes × ^{15}N recovery form first (C) and second (D) growing seasons. Canonical axes are labeled with percentage of total variance (RDA_1 = main variation; RDA_2 = remaining variation). Arrows indicate correlations between variables. The significance of these correlations was assessed by Monte Carlo permutation test with 999 permutations. Significant variables ($p \leq 0.05$) are indicated by an asterisk. Color dashed lines indicate significant ($p \leq 0.05$) clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). First season variables are represented by red color, whereas second season are represented by green color (A and B). SOC = soil organic carbon; ^{15}N rec. = ^{15}N recovery; SDW = stover dry weight; GDW = grain dry wight; RDW = ruzigrass biomass dry weight; *16S rRNA bac. = 16S rRNA bacteria*; *16S rRNA arc*. = *16S rRNA archaea*.

Considering the role of soil fertility on soil microbial genes, PERMANOVA analysis also segregated the treatments into four distinct groups, although control and PG treatments showed a certain degree of similarity. The sum of the axes indicated that soil fertility explained 70% of all variation in the abundance of soil microbial genes, with the main soil factors responsible for this change being soil pH, and SOC content (Figure 6B). The highest abundances of denitrifying genes (*nirK* and *nosZ*) correlated positively with the control and PG treatments, as well as the *AmoA* gene during the soybean season. On the other hand, the soil factors indicated a greater abundance of

total bacteria and total archaea in treatments with higher soil fertility (L and LPG). The same occurred for ammonia–oxidizing microbe genes (*AmoA* and *AmoB*) during the maize season. Our third RDA revealed how soil microbial genes were responsible for modulating ^{15}N recovery in the maize + ruzigrass season (Fig. 6C). Axis 1 was responsible for explaining 93% of the variation that occurred. PERMANOVA segregated treatments into four groups, indicating differences between them. In addition, the highest ^{15}N losses in the maize + ruzigrass season were positively correlated with denitrification genes from the control treatment. On the other hand, the highest abundances of microbes (bacteria and archaea) and of nitrifying genes correlated with the highest ^{15}N recovery by maize and ruzigrass growing under L and especially LPG treatments. Total archaea, *AmoA*, *AmoB*, *nirK*, and *nosZ* were the main soil microbial genes that modulated the ^{15}N–fertilizer utilization during the maize + ruzigrass season. Lastly, our results by combining soil microbial genes with ^{15}N recovery by soybean in the second season indicated that the treatments were different from each other (PERMANOVA analysis) and that the sum of the axes explained 62% of all the variation that occurred. In this scenario, only the *nirK* gene participated significantly in the modulation of ^{15}N recovery responses, especially when associated with control and PG treatments. Furthermore, the highest ^{15}N recovery by soybean also occurred in these treatments. On the other hand, the largest losses of ^{15}N–fertilizer in the second season occurred in L and LPG–amended soils.

5.4 DISCUSSION

5.4.1 Soil profile fertility

Surface–applied amendments in no–till proved to be a feasible practice for reducing subsoil acidity and improving soil fertility over time. Here, we present the long–term construction of subsoil fertility after four applications of L and/or PG amendment over 17 years. The results of this study refer to the third year after the last reapplication of these soil amendments. Subsoil acidity was sharply reduced through liming treatments (L and LPG); however, combining L with PG (LPG) increased Ca^{2+} concentrations in all soil layers, especially in-depth. Exchangeable Ca^{2+} concentration at deeper layers proved to be essential for deep rooting to increase nutrient and water

uptake (Bossolani et al., 2021c), as well as reducing the risk of water deficit during dry spell periods (Bossolani et al., 2021a).

Sole application of PG did not change soil pH, although it increased Ca^{2+} concentrations in the soil profile compared with control. The effects of PG alone are small compared to L; however, this amendment potentiates the effects of L when applied in combination, but not as a substitute (Crusciol et al., 2019). When PG is applied to soil, it quickly dissociates into SO_4^{2-} and Ca^{2+} (Zoca and Penn, 2017). In addition to the fertilizing effect (S and Ca supply), PG also reduces the Al^{3+} toxicity (Tiecher et al., 2018). Additionally, $SO4^{2-}$ creates ionic pairs with exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, NH_4^+), displacing them to deeper layers (Ernani et al., 2006; Tiecher et al., 2018; Zoca and Penn, 2017). Exchangeable Mg^{2+} concentration also increased along with soil profile after liming (L and LPG), regardless of the PG combination. Low Mg^{2+} availability is limiting for crop development, but fertilization programs do not include this nutrient in conventional NPK formulas. For this reason, Mg^{2+} is often associated with a “forgotten element” (Cakmak and Kirkby, 2008). Acidic soils are poor in Mg^{2+}, which is essential in numerous metabolic plant processes (Jaghdani et al., 2021). Liming is the most viable way for Mg^{2+} fertilization, making it less limiting in intensive crop production systems (Li et al., 2019). The ability of SO_4^{2-} to create ionic pairs with Mg^{2+} (Zoca and Penn, 2017) increased the concentration of this element in deep layers from LPG–amended soil when compared with L alone. Soil organic C content was also changed by long–term effects of L and LPG treatments in all soil layers. Soils with adequate fertility management provide higher above– and belowground crop residues (Bossolani et al., 2021a; Costa et al., 2018), leading to increases in SOC over time (Carmeis Filho et al., 2017c; Inagaki et al., 2017).

5.4.2 Aboveground dry matter yield and fate of ^{15}N–labeled fertilizer

As a result of improved soil fertility by soil amendment applications, the aboveground dry matter production of maize, ruzigrass, and soybean also increased. In general, these crops have a low tolerance to acidic soils with high Al^{3+} concentrations (Rao et al., 1993). Several studies have demonstrated the importance of liming, especially when combined with PG in increasing biomass and grain yield (Bossolani et al., 2022, 2021a; Costa et al., 2018; Crusciol et al., 2019; Soratto and Crusciol, 2008). Although there was no difference between L and LPG in straw

production, maize and soybean grain yields increased in LPG–amended soil. The improvement in these attributes is due to the improvements in soil fertility (*i.e.*, higher SOC, Ca^{2+}, Mg^{2+}, and SO_4^{2-}–S concentrations), and the reduction of Al^{3+} concentration, as supported by RDA, which showed a positive correlation between stover and grain yield with soil fertility parameters. The increase in Ca^{2+} levels in deeper layers is strongly linked to the rooting depth, which improves soil resources uptake by crops (Costa et al., 2018; Ritchey et al., 1982). Our results demonstrate the importance of combing L to PG (LPG treatment) in annual crop rotations to obtain high straw (aiming no–till system maintenance) and grain yield.

The cascading effects of soil amendments in soil fertility, and crop growth and yield changed the ^{15}N fate. LPG–amended soil not only increased ^{15}N recovery by maize grains, but also increased ^{15}N accumulation in ruzigrass biomass. While the ^{15}N in the ruzigrass biomass and maize stover act as N pools that potentially return to system (^{15}N remaining data) through crop residue decomposition, N in grains is exported after harvest. On the other hand, all N that is contained in plant tissues becomes less susceptible to losses to the environment, either by denitrification or leaching (Heijboer et al., 2016; Zhou et al., 2020). Increasing the recovery of N–fertilizers by the crops guarantees an increase in the yield of the crops, as well as an environmentally safe agriculture. This fact is reinforced by the results of unrecovered ^{15}N after maize grown. Soils managed with L and LPG had the lowest N losses, whereas the control (no amendments applied) led to the highest ^{15}N losses.

The soybean-maize (intercropped with forage grasses) rotation is an agricultural system widely used in tropical regions, primarily in Brazil (Ceccon et al., 2013). Interestingly, the ^{15}N recovery by maize and ruzigrass shows an inverse trend to that obtained in soybean cultivated in rotation. The yield–scale ^{15}N recovery for each season reinforces this pattern. Overall, ~70% of the total N accumulated by soybean comes from biological N fixation [BNF; (Ciampitti and Salvagiotti, 2018)], but the ^{15}N recovery was higher in the control and PG treatments. This effect is underpinned by the following rationale: (*i*) the smaller amount of ^{15}N–fertilizer remaining in the soil from L and LPG treatments, and/or more likely (*ii*) liming increased the efficiency of BNF (Alves et al., 2021; Andrade et al., 2002), reducing dependence on soil fertilizer-derived ^{15}N. Several studies have shown that changes in soil pH by liming have led to a shift in Rhizobia species composition (Andrade et al., 2002; Zhalnina et al., 2013).

According to these authors, the abundance of N–fixing Rhizobia species markedly reduced in highly acidic soil.

5.4.3 Distribution of ^{15}N–fertilizer along soil profile and soil microbial genes

Soil retention data indicated a high concentration of ^{15}N–fertilizer in deep layers in PG–amended soil, whereas the smallest amounts of ^{15}N–labeled fertilizer occurred in L and LPG treatments. Liming has been associated as a catalyst in numerous soil biological processes (Guo et al., 2019; Liu et al., 2018). The cascading effects of liming on soil fertility can drastically affect soil N transformation processes by microorganisms (Bossolani et al., 2020), regulating the release of plant-available N, as well as losses through groundwater and/or to the atmosphere (Holland et al., 2018; Yang et al., 2021).

Liming and, especially, L combined with PG (LPG) triggered positive effects in the total bacteria and archaea gene abundances. The increase in soil fertility is often correlated with changes in soil microbial communities (Bossolani et al., 2021b; Holland et al., 2018). In response to the increase in bacterial and archaeal populations, the abundance of N–cycle genes also increased (Fig. S1). High abundances of ammonia–oxidizing microbial genes (*AmoB* and, especially *AmoA*) were found in L and LPG treatments. Archaea are the most abundant microorganisms responsible for nitrification in agricultural soils with low NH4+-N content, while nitrifying bacteria, despite being low in abundance, outcompeted with archaea at high NH4+-N fertilization (Rütting et al., 2021). Our third RDA revealed the high influence of archaea (16S rRNA and *AmoA* abundances) during the maize season, shaping the ^{15}N recovery responses. Increasing nitrifying microorganisms by liming suggests that fertilizer-derived NH_4^+ is rapidly converted to NO_3^- (Beeckman et al., 2018). Numerous studies have reported an increase in the genes of nitrifying microorganisms in soils managed with liming (Bossolani et al., 2020; Liu et al., 2018). Under no biotic or abiotic impediments to plant growth, NO_3^- generated by nitrification is taken up by the crops; conversely, in case of low uptake rates (*e.g.*, incipient root system) or excessive rainfall, there is a risk of NO_3^- leaching (Holland et al., 2018). The high yields of biomass and grains in the L and LPG treatments, combined with the low amount of ^{15}N–fertilizer in deep layers indicate an intense N uptake by the crops. However, high amounts of ^{15}N were found in the soil of PG treatment. This result is probably due to the acidic conditions, which reduce the nitrification rate (Beeckman et al., 2018).

Additionally, the SO_4^{2-} generated from PG dissociation in the soil possibly formed an ion pair with NH_4^+, resulting in zero–charged ammonium sulfate [$(NH_4)_2SO_4$], and then leached to deeper soil layers before being absorbed by the crops. Bossolani et al. (2021a) showed that root growth in control and PG–amended soil was shallowest than in L and LPG treatments. These authors also demonstrated that the high concentration of Al^{3+} and the low concentration of Ca^{2+} in depth were the main soil factors responsible for the lower root growth. The leaching to layers beyond the root zone turns N unavailable to plants and is eventually carried to groundwater. Interestingly, smaller amounts of ^{15}N–fertilizer were found in deep layers of control treatment when compared to PG. Unamended acidic soil also showed lower abundances of the *AmoB* and *AmoA* genes compared to limed treatments (L and LPG). Although these results indicate low nitrification rates, the control presented the highest abundance of microbial genes related to denitrification (*nirK* and *nosZ*), as supported by the third and fourth RDAs. The lower N uptake rate by crops increases the availability of mineral N (NH_4^+ and NO_3^-), providing substrates for denitrifying populations (Zhou et al., 2020), thus favoring conditions for N_2O and N_2 production (Harter et al., 2014).

Lastly, our multifaceted approach by combining different sets of variables indicated that soil pH and SOC were the main soil factors responsible for increasing the abundance of total bacteria and archaea, as well as the genes of nitrifying microorganisms, whereas reducing the abundance of denitrifiers. Liming triggers soil buffering processes by changing soil pH that increase the soil nutrient availability (Holland et al., 2018), which are reflected in an increase in the yield capacity of crops (Costa et al., 2018). The high return of plant residues in fertile soils leads to increased plant growth (root and shoot) that usually results in higher SOC contents in the long–term (Carmeis Filho et al., 2017c; Inagaki et al., 2017). This set of changes shapes the soil microbial communities by providing substrate (C, N and other nutrients) for their growth (Hemkemeyer et al., 2021). The biggest changes in soil fertility and crop yields occurred through liming, but PG further potentiates these effects. Therefore, managing the soil with soil amendments, such as LPG, is an essential tool to trigger synergistic effects between high soil quality, crop yield, and the use efficiency of N fertilizers, aiming to increase the sustainability of agricultural systems.

5.5 CONCLUSIONS

Our study provides important information about changes in soil fertility and crop yield from a no–till crop rotation system managed with lime and phosphogypsum applications, and the effect of these changes on ^{15}N–labeled fertilizer recovery. Our findings indicated that there is a greater abundance of genes related to nitrification in limed soils, especially when combined with phosphogypsum, however, there is also greater ^{15}N–fertilizer recovery in these treatments due to greater uptake and growth by maize and ruzigrass. On the other hand, soybean plants recovered greater amount of ^{15}N–fertilizer from control and exclusive phosphogypsum treatments. In addition, our results suggest that in the absence of liming, there is a reduction in the abundance of genes related to nitrification, and in this situation, the application of high phosphogypsum rates can trigger greater leaching of NH_4^+ to deep layers. Future research should focus on quantifying the emission of gases from denitrification and correlating it with the soil microbial community assessed by DNA sequencing. Our results generated information that is vital for farmers to develop and implement programs that increase the use efficiency of N–fertilizers for increased yield, lowering cost and decreasing environmental damage.

Acknowledgements

This study was supported by São Paulo Research Foundation (FAPESP; grant number 2018/11063–7 and 2019/12764–1) and the National Council for Scientific and Technological Development (CNPq; Universal Research Project: 421637/2018–8). In addition, CACC received a research productivity fellowships (PQ) from the CNPq (310535/2021-2).

REFERENCES

Alves, L.A., Ambrosini, V.G., Denardin, L.G. de O., Flores, J.P.M., Martins, A.P., Filippi, D., Bremm, C., Carvalho, P.C. de F., Farias, G.D., Ciampitti, I.A., Tiecher, T., 2021. Biological N2 fixation by soybeans grown with or without liming on acid soils in a no-till integrated crop-livestock system. Soil Tillage Res. 209. https://doi.org/10.1016/j.still.2020.104923

Anderson, M.J., 2005. Permutational multivariate analysis of variance. Dep. Stat. Univ. Auckland, Auckl. 26, 32–46.

Andrade, D.S., Murphy, P.J., Giller, K.E., 2002. Effects of liming and legume/cereal

cropping on populations of indigenous rhizobia in an acid Brazilian Oxisol. Soil Biol. Biochem. 34, 477–485. https://doi.org/10.1016/S0038-0717(01)00206-1

Ashitha, A., Rakhimol, K.R., Mathew, J., 2021. Fate of the conventional fertilizers in environment, in: Controlled Release Fertilizers for Sustainable Agriculture. Elsevier, pp. 25–39.

Bardsley, C.E., Lancaster, J.D., 1960. Determination of reserve sulfur and soluble sulfates in soils. Soil Sci. Soc. Am. J. 24, 265–268. https://doi.org/https://doi.org/10.2136/sssaj1960.03615995002400040015x

Beeckman, F., Motte, H., Beeckman, T., 2018. Nitrification in agricultural soils: impact, actors and mitigation. Curr. Opin. Biotechnol. 50, 166–173. https://doi.org/10.1016/j.copbio.2018.01.014

Blake, G.R., Hartge, K.H., 1986. Bulk density. Methods soil Anal. Part 1 Phys. Mineral. methods 5, 363–375.

Bossolani, J.W., Alexandre, C., Crusciol, C., Moretti, L.G., Garcia, A., Bernart, L., Vilela, R.G., Caires, E.F., Jorge, T., Amado, C., Calonego, J.C., Rodrigues, A., Alexandre, C., Crusciol, C., 2022. Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics. Agron. Sustain. Dev. 9, 1–19. https://doi.org/https://doi.org/10.1007/s13593-022-00765-9 (2022)

Bossolani, J.W., Crusciol, C.A.C., Garcia, A., Moretti, L.G., Portugal, J.R., Rodrigues, V.A., Fonseca, M. de C. da, Calonego, J.C., Caires, E.F., Amado, T.J.C., Reis, A.R. dos, 2021ª. Long-term lime and phosphogypsum amended-soils alleviates the field drought effects on carbon and antioxidative metabolism of maize by improving soil fertility and root growth. Front. Plant Sci. 12, 1437. https://doi.org/10.3389/fpls.2021.650296

Bossolani, J.W., Crusciol, C.A.C., Leite, M.F.A., Merloti, L.F., Moretti, L.G., Pascoaloto, I.M., Kuramae, E.E., 2021b. Modulation of the soil microbiome by long-term Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. Soil Biol. Biochem. 156, 108188. https://doi.org/10.1016/j.soilbio.2021.108188

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375, 114476. https://doi.org/10.1016/j.geoderma.2020.114476

Bossolani, J.W., Crusciol, C.A.C., Portugal, J.R., Moretti, L.G., Garcia, A., Rodrigues, V.A., Fonseca, M. de C. da, Bernart, L., Vilela, R.G., Mendonça, L.P., Reis, A.R., 2021c. Long-term liming improves soil fertility and soybean root growth, reflecting improvements in leaf gas exchange and grain yield. Eur. J. Agron. 128, 126308. https://doi.org/10.1016/j.eja.2021.126308

Brody, J.R., Kern, S.E., 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques 36, 214–216. https://doi.org/https://doi.org/10.2144/04362BM02

Caires, E.F., Corrêa, J.C.L., Churka, S., Barth, G., Garbuio, F.J., 2006. Surface

application of lime ameliorates subsoil acidity and improves root growth and yield of wheat in an acid soil under no-till system. Sci. Agric. 63, 502–509. https://doi.org/10.1590/S0103-90162006000500013

Caires, E.F., Guimarães, A.M., 2018. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. Agron. J. 110, 1987–1995. https://doi.org/10.2134/agronj2017.11.0642

Cakmak, I., Kirkby, E.A., 2008. Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol. Plant. 133, 692–704. https://doi.org/10.1111/j.1399-3054.2007.01042.x

Carmeis Filho, A.C.A., Crusciol, C.A.C., Castilhos, A.M., 2017a. Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. J. Agric. Sci. 155, 1093–1112. https://doi.org/10.1017/S0021859617000235

Carmeis Filho, A.C.A., Crusciol, C.A.C., Guimarães, T.M., Calonego, J.C., Mooney, S.J., 2017b. Impact of amendments on the physical properties of soil under tropical long-term no till conditions. PLoS One 12, 27959897. https://doi.org/10.1371/journal.pone.0173011

Carmeis Filho, A.C.A., Penn, C.J., Crusciol, C.A.C., Calonego, J.C., 2017c. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. Agric. Ecosyst. Environ. 241, 11–23. https://doi.org/10.1016/j.agee.2017.02.027

Ceccon, G., Staut, L.A., Sagrilo, E., Machado, L.A.Z., Nunes, D.P., Alves, V.B., 2013. Legumes and forage species sole or intercropped with corn in soybean-corn succession in midwestern Brazil. Ver. Bras. Ciência do Solo 37, 204–212. https://doi.org/10.1590/s0100-06832013000100021

Ciampitti, I.A., Salvagiotti, F., 2018. New insights into soybean biological nitrogen fixation. Agron. J. 110, 1185–1196. https://doi.org/10.2134/agronj2017.06.0348

Costa, C.H.M., Carmeis Filho, A.C.A., Crusciol, C.A.C., Soratto, R.P., Guimarães, T.M., 2018. Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. Crop Pasture Sci. 69, 488–505. https://doi.org/10.1071/CP17233

Crusciol, C.A.C., Bossolani, J.W., Portugal, J.R., Moretti, L.G., Momesso, L., de Campos, M., Costa, N.R., Volf, M.R., Calonego, J.C., Rosolem, C.A., 2022. Exploring the synergism between surface liming and nitrogen fertilization in no-till system. Agron. J. 113, 1–10.

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr. Cycl. Agroecosystems 115, 347–372. https://doi.org/10.1007/s10705-019-10017-0

Ernani, P.R., Miquelluti, D.J., Fontoura, S.M. V, Kaminski, J., Almeida, J.A., 2006. Downward movement of soil cations in highly weathered soils caused by addition of gypsum. Commun. Soil Sci. Plant Anal. 37, 571–586.

Fageria, N.K., Nascente, A.S., 2014. Management of soil acidity of South American soils for sustainable crop production, Advances in Agronomy. Elsevier. https://doi.org/10.1016/B978-0-12-802139-2.00006-8

Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development.

Garbuio, F.J., Jones, D.L., Alleoni, L.R.F., Murphy, D. V., Caires, E.F., 2011. Carbon and Nitrogen Dynamics in an Oxisol as Affected by Liming and Crop Residues under No-Till. Soil Sci. Soc. Am. J. 75, 1723–1730. https://doi.org/10.2136/sssaj2010.0291

Guo, A., Ding, L., Tang, Z., Zhao, Z., Duan, G., 2019. Microbial response to CaCO3 application in an acid soil in southern China. J. Environ. Sci. 79, 321–329. https://doi.org/10.1016/j.jes.2018.12.007

Harter, J., Krause, H.M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., Kappler, A., Behrens, S., 2014. Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J. 8, 660–674. https://doi.org/10.1038/ismej.2013.160

Heanes, D. L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Commun Soil Sci Plant Anal, 10, 191-1213.

Heijboer, A., ten Berge, H.F.M., de Ruiter, P.C., Jørgensen, H.B., Kowalchuk, G.A., Bloem, J., 2016. Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15N tracer-based approach. Appl. Soil Ecol. 107, 251–260. https://doi.org/10.1016/j.apsoil.2016.06.009

Hemkemeyer, M., Schwalb, S.A., Heinze, S., Joergensen, R.G., Wichern, F., 2021. Functions of elements in soil microorganisms. Microbiol. Res. 126832. https://doi.org/10.1016/j.micres.2021.126832

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. 610–611, 316–332. https://doi.org/10.1016/j.scitotenv.2017.08.020

Inagaki, T.M., de Moraes Sá, J.C., Caires, E.F., Gonçalves, D.R.P., 2017. Why does carbon increase in highly weathered soil under no-till upon lime and gypsum use? Sci. Total Environ. 599–600, 523–532. https://doi.org/10.1016/j.scitotenv.2017.04.234

Jaghdani, S.J., Jahns, P., Tränkner, M., 2021. Mg deficiency induces photo-oxidative stress primarily by limiting CO2 assimilation and not by limiting photosynthetic light utilization. Plant Sci. 302. https://doi.org/10.1016/j.plantsci.2020.110751

Levene, H., 1960. Robust tests for equality of variances, in: Olkin, I., Ghurye, S.G., Hoeffding, W., Madow, W.G., Mann, H.B. (Eds.), Contributions to Probability and Statistics: Essays in …. Stanford University Press, pp. 278–292.

Li, Y., Cui, S., Chang, S.X., Zhang, Q., 2019. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. J. Soils Sediments 19, 1393–1406.

https://doi.org/10.1007/s11368-018-2120-2

Liu, J., Liu, M., Wu, M., Jiang, C., Chen, X., Cai, Z., Wang, B., Zhang, J., Zhang, T., Li, Z., 2018. Soil pH rather than nutrients drive changes in microbial community following long-term fertilization in acidic Ultisols of southern China. J. Soils Sediments 18, 1853–1864. https://doi.org/10.1007/s11368-018-1934-2

Liu, X.Y., Rezaei Rashti, M., Esfandbod, M., Powell, B., Chen, C.R., 2018. Liming improves soil microbial growth, but trash blanket placement increases labile carbon and nitrogen availability in a sugarcane soil of subtropical Australia. Soil Res. 56, 235–243. https://doi.org/10.1071/SR17116

Meng, C., Tian, D., Zeng, H., Li, Z., Yi, C., Niu, S., 2019. Global soil acidification impacts on belowground processes. Environ. Res. Lett. 14, 074003. https://doi.org/10.1088/1748-9326/ab239c

Nelson, L.S., 1998. The Anderson-Darling test for normality. J. Qual. Technol. 30, 298.

Rao, I.M., Zeigler, R.S., Vera, R., Sarkarung, S., 1993. Selection and breeding for acid-soil tolerance in crops. Bioscience 43, 454–465.

Ritchey, K.D., Silva, J.E., Costa, U.F., 1982. Calcium deficiency in clayey B horizons of savanna oxisols. Soil Sci. 133, 378–382.

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1993. How a corn plant develops. Special Report n. 48. Iowa State University of Science and Technology. Coop. Ext. Serv. Ames, IA, USA.

Rütting, T., Schleusner, P., Hink, L., Prosser, J.I., 2021. The contribution of ammonia-oxidizing archaea and bacteria to gross nitrification under different substrate availability. Soil Biol. Biochem. 160, 108353. https://doi.org/10.1016/j.soilbio.2021.108353

Santos, D.R., 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

Shi, P., Zhang, Y., Hu, Z., Ma, K., Wang, H., Chai, T., 2017. The response of soil bacterial communities to mining subsidence in the west China aeolian sand area. Appl. Soil Ecol. 121, 1–10. https://doi.org/10.1016/j.apsoil.2017.09.020

Soil Survey Staff, 2014. Keys to Soil Taxonomy. USDA - Natural Resources Conservation Service, Washington, DC, p. 360.

Soratto, R.P., Crusciol, C.A.C., 2008. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. Agron. J. 100, 261–270. https://doi.org/10.2134/agronj2007.0120

Sumner, M.E., Noble, A.D., 2003. Soil acidification: the world story. Handb. soil acidity. Marcel Dekker, New York 1–28.

Tiecher, T., Pias, O.H. de C., Bayer, C., Martins, A.P., Denardin, L.G. de O., Anghinoni, I., 2018. Crop response to gypsum application to subtropical soils

under no-till in Brazil: A systematic review. Ver. Bras. Cienc. do Solo 42, 1–17. https://doi.org/10.1590/18069657rbcs20170025

Tiritan, C.S., Büll, L.T., Crusciol, C.A.C., Carmeis Filho, A.C.A., Fernandes, D.M., Nascente, A.S., 2016. Tillage system and lime application in a tropical region: Soil chemical fertility and corn yield in succession to degraded pastures. Soil Tillage Res. 155, 437–447. https://doi.org/10.1016/j.still.2015.06.012

van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendações da adubação e calagem para o Estado de São Paulo. Instituto Agronômico/Fundação IAC Campinas.

van Raij, B., Quaggio, J.A., Cantarella, H., Abreu, C.A., 2001. Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. Análise química para avaliação da Fertil. solos Trop. 5–39.

Weligama, C., Sale, P.W.G., Conyers, M.K., Liu, D.L., Tang, C., 2010. Nitrate leaching stimulates subsurface root growth of wheat and increases rhizosphere alkalisation in a highly acidic soil. Plant Soil 328, 119–132.

Yang, F., Zhang, Z., Barberán, A., Yang, Y., Hu, S., Guo, H., 2021. Nitrogen-induced acidification plays a vital role driving ecosystem functions: Insights from a 6-year nitrogen enrichment experiment in a Tibetan alpine meadow. Soil Biol. Biochem. 153. https://doi.org/10.1016/j.soilbio.2020.108107

Zhalnina, K., De Quadros, P.D., Gano, K.A., Davis-Richardson, A., Fagen, J.R., Brown, C.T., Giongo, A., Drew, J.C., Sayavedra-Soto, L.A., Arp, D.J., Camargo, F.A.O., Daroub, S.H., Clark, I.M., McGrath, S.P., Hirsch, P.R., Triplett, E.W., 2013. Ca. nitrososphaera and bradyrhizobium are inversely correlated and related to agricultural practices in long-term field experiments. Front. Microbiol. 4, 1–13. https://doi.org/10.3389/fmicb.2013.00104

Zhou, W., Jones, D.L., Hu, R., Clark, I.M., Chadwick, D.R., 2020. Crop residue carbon-to-nitrogen ratio regulates denitrifier N2O production post flooding. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-020-01462-z

Zoca, S.M., Penn, C., 2017. An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. Adv. Agron. 144, 1–44. https://doi.org/10.1016/bs.agron.2017.03.001

SUPPLEMENTARY MATERIAL

Table S1. Crop history and the treatment application scheme throughout the experimental period (2002–2020)

Crop season	Sowing	Crops	Harvest	Treatment application
2002/2003	Nov.	*Oriza sativa*	Mar.	Lime: 2.7 Mg ha^{-1} (71% ECCE†) Phosphogypsum: 2.1 Mg ha^{-1}
	Apr.	*Avena strigosa*	Aug	
2003/2004	Jan.	*Phaseolus vulgaris*	Apr.	–
	Apr.	*Avena strigosa*	Aug	
2004/2005	Nov.	*Arachis hypogaea*	Apr.	Lime: 2.0 Mg ha^{-1} (71% ECCE) Phosphogypsum: 2.1 Mg ha^{-1}
	Apr.	*Avena sativa*	Sept.	
2005/2006	Nov.	*Arachis hypogaea*	Apr.	–
	May	*Avena sativa*	Sept.	
2006/2007	Feb.	*Zea mays* + *Urochloa brizantha*	Apr.	–
	-	*Urochloa brizantha*	–	
2007/2008	Dec.	*Zea mays* + *Urochloa brizantha*	Mar.	–
	-	*Urochloa brizantha*	–	
2008/2009	Dec.	*Glycine max*	Apr.	–
	Jun.	*Avena strigosa*	Oct.	
2009/2010	Oct.	*Glycine max*	Feb.	–
	Mar.	*Sorghum vulgare*	July	
2010/2011	Nov.	*Zea mays*	Apr.	Lime: 2.0 Mg ha^{-1} (88% ECCE) Phosphogypsum: 2.1 Mg ha^{-1}
	Apr.	*Crambe abyssinica*	Aug.	
	Sept.	*Vigna unguiculata*	Dec	
2011/2012	Nov.	*Zea mays*	Feb.	–
	May	*Crambe abyssinica*	Aug.	
	Sept.	*Vigna unguiculata*	Jan.	
2012/2013	Jan.	*Pennisetum glaucum*	Apr.	–
	Apr.	*Triticum aestivum*	Aug.	
2013/2014	Nov.	*Phaseolus vulgaris*	Feb.	–
	Mar.	*Triticum aestivum*	Jun.	
2014/2015	Dec.	*Phaseolus vulgaris*	Mar.	–
	Mar.	*Urochloa brizantha*	–	
2015/2016	-	*Urochloa brizantha*	–	–
	-	*Urochloa brizantha*	–	
2016/2017	Oct.	*Glycine max*	Mar.	Lime: 13 Mg ha^{-1} (69% ECCE) Phosphogypsum: 10 Mg ha^{-1}
	Mar.	*Zea mays* + *Urochloa ruziziensis*	July	
2017/2018	Oct.	*Glycine max*	Mar.	–
	Mar.	*Zea mays* + *Urochloa ruziziensis*	July	
2018/2019	Oct.	*Glycine max*	Mar.	–
	Mar.	*Zea mays* + *Urochloa ruziziensis*	Aug.	
2019/2020	Oct.	*Glycine max*	Mar.	–
	Mar.	*Zea mays* + *Urochloa ruziziensis*‡	July	
2020/2021	Nov.	*Glycine max*	Mar.	–

†ECCE: effective calcium-carbonate equivalent; ‡^{15}N-labeled fertilizer application;

Table S2 - Description of primers, standards DNA and amplification conditions that were used in qPCR analysis.

	Target Genes	*Primers*	*DSMZ* code†	Sequence (5'- 3')	Fragment size (pb)	Reference	Conditions of Amplification
Phylogenetic Markers	*16S rRNA Bacteria*	Eub 338f Eub 518r	17167	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	180	Bakke et al. (2011)	95ºC - 10 min, 40 cycles, 94ºC - 15 s, 56ºC - 30 s e 72 ºC - 45 s
	16S Archaea	519F 915R	Environmental DNA‡	CAGCCGCCGCGGTAA GTGCTCCCCCGCCAATTCCT	397	Klindworth et al. (2013)	95ºC - 10 min, 40 cycles, 95ºC - 30 s, 57ºC - 30 s e 72 ºC -50 s
	nifH	-	17167	AAAGGYGGWATCGGYAARTCCACCAC TTGTTSGCSGCRTACATSGCCATCAT	457	Wallenstein and Vilgalys, (2005)	95ºC - 5 min, 40 cycles, 95 ºC - 30 s, 59ºC - 30 s e 72ºC - 1 min e 72º C - 1 min
Functional genes associated with the N cycle	*amoA of Bacteria*	*amoB 1F amoB 2F*	28437	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	491	Rotthauwe et al. (1997)	95ºC - 10 min, 40 cycles, 95ºC - 45 s, 60ºC - 45 s e 72ºC - 45 s
	amoA of Archaea	*amoA1F amoA 2F*	Environmental DNA	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	635	Francis et al. (2005)	95ºC - 5 min, 40 cycles, 95ºC - 40 s, 56ºC - 30 s e 72ºC - 60 s
	nirK	*876 F 1040 R*	30026	ATYGGCGGVCAYGGCGA GCCTCGATCAGRTTRTGGTT	520	Henry et al. (2004)	95ºC - 10 min, 6 cycles, 95ºC - 15 s, 63ºC - 30 s e 72ºC - 40 s, 95ºC - 30 s, 38 cycles, 58ºC - 30s, 72ºC - 40 s
	nosZ	*nosZ* 2F *nosZ 2R*	17167	CGCRACGGCAASAAGGTSMSSGT CAKRTGCAKSGCRTGGCAGAA	267	Henry et al. (2006)	95 ºC - 15s, 60 ºC - 40s, 72 ºC - 40s

†Reference code of cell catalog of the Leibniz Institute DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH*), used in the construction of the standard curves for qPCR analysis.

‡ The PCR products were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, UK)

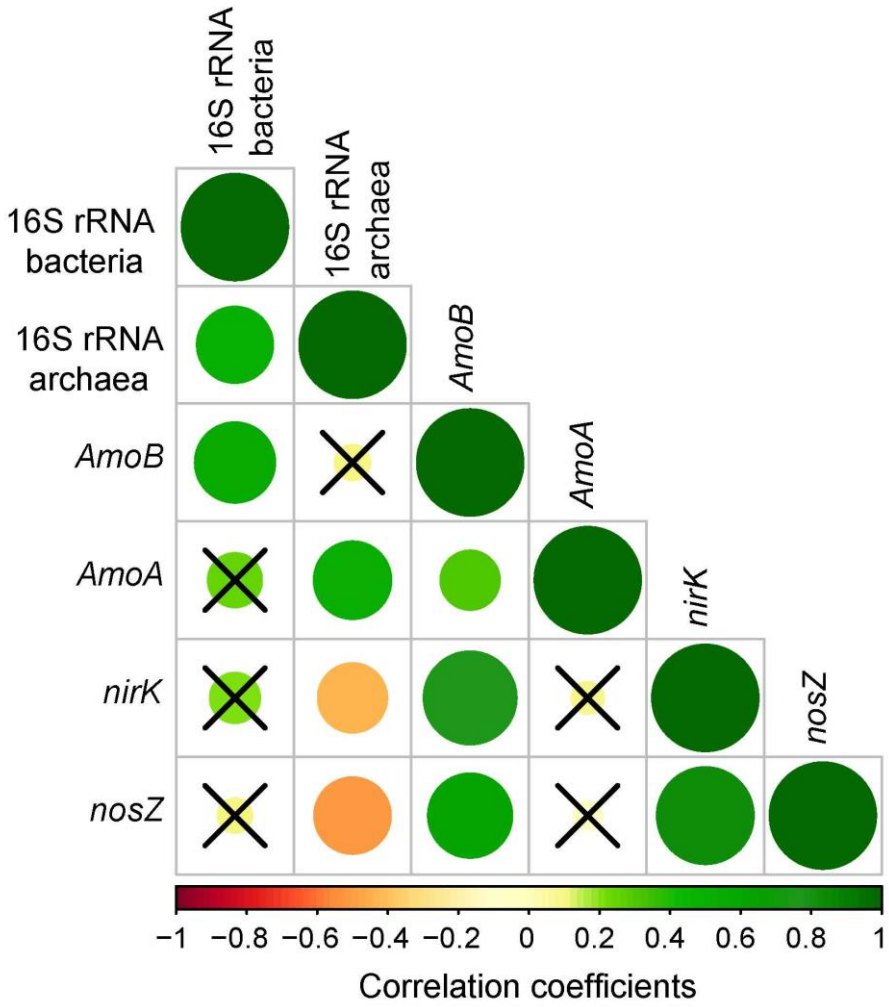


Figure S1. Heatmap of the Pearson's correlation coefficients among the total abundance of N–cycle functional genes. Only significant correlations ($p \leq 0.05$) are not marked with "×".

GENERAL CONCLUSIONS

Our results advances the understanding of the dynamics of surface lime application and the combined effect of lime and phosphogypsum in the different aspects that make up a long-term tropical no-till system. Our results showed that the application of lime alone and especially in combination with phosphogypsum improves soil fertility up to 1 m deep, culminating in increased root growth of soybean and maize crops. As a result, both crops increased the leaf nutrient concentrations, reflecting an improvement in the concentration of leaf photosynthetic pigments, and gas exchange (net photosynthetic rate, stomatal conductance, substomatal concentration of CO_2, leaf transpiration and water use efficiency), and on the activities of Rubisco and SuSy enzymes. Interestingly, when studying the water balance during the soybean and maize growing cycle, we confirmed periods of water scarcity, and when lime and phosphogypsum were applied, drought stress (measured through antioxidant enzymes and lipid peroxidation) reduced. Crop grain yield was also increased with liming, and even more expressive results occurred by the combination of lime and phosphogypsum. Interestingly, soil microbiology highly contributed to the understanding of the dynamics of soil-plant relationship of this study. The productive responses of soybean and maize did not occur only due to changes in the soil chemical properties. Deepening the understanding of tropical agricultural systems altered by the application of lime and phosphogypsum, our results revealed that the joint application of these inputs changed the soil chemical properties in tropical intercropping system managed under no-till, increasing the relative abundance of total prokaryotes (archaea and bacteria), modulating genes related to the nitrogen cycle. Exchangeable Ca^{2+} and Mg^{2+} were the main nutrients related to the increase of genes related to nitrogen fixation and to the reduction of the relative abundance of genes related to nitrification and denitrification (even though the total abundance increased), for both soil and rhizospheres of maize and ruzigrass. In addition, the synergism between lime and phosphogypsum also increased the quantity and quality of soil organic matter, mainly by altering the relationship between bacteria and fungi communities of the soil. Interestingly, we found that soil improvers act by modifying soil quality at a hierarchical level as follows: soil fertility > microbiome (fungi + bacteria) > chemical fractions of soil organic matter > soil physics > physical fractions of soil organic matter. Finally, our results also revealed that the combined application of these inputs has a strong

influence on the selection of microorganisms that are not abundant in the soil (rare community), reflecting a potential increase in the multifunctionality of the soil. Our results also showed that the increase in the abundance of related genes in soils managed with lime and phosphogypsum favors the recovery of ^{15}N fertilizer, which strongly reduces the losses of nitrogen fertilizer. On the other hand, we saw that the isolated use of phosphogypsum has a potential to leaching N to deep layers. In the absence of the management with lime and phosphogypsum (control treatment), the abundance of genes related to denitrification has increased, which indicates a high potential for denitrification in these soils, increasing even more the N losses in the system.

REFERENCES

BADALUCCO, L.; GREGO, S.; DELL'ORCO, S.; NANNIPIERI, P. Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (Picea abies L.). **Biology and Fertility of Soils**, v. 14, p. 76-83, 1992.

BOSSOLANI, J. W.; CRUSCIOL, C. A. C.; LEITE, M. F. A.; MERLOTI, L. F.; MORETTI, L. G.; PASCOALOTO, I. M.; KURAMAE, E. E. Modulation of the soil microbiome by long-term Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. **Soil Biology and Biochemistry**, v. 156, 108188, 2021a. DOI: https://doi.org/10.1016/j.soilbio.2021.108188

BOSSOLANI, J. W.; CRUSCIOL, C. A. C.; GARCIA, A.; MORETTI, L. G.; PORTUGAL, J. R.; RODRIGUES, V. A.; FONSECA, M. C.; CALONEGO, J. C.; CAIRES, E. F.; AMADO, T. J. C.; REIS, A. R. D. Long-term lime and phosphogypsum amended-soils alleviates the field drought effects on carbon and antioxidative metabolism of maize by improving soil fertility and root growth. **Frontiers in Plant Science**, 1437, 2021b. https://doi.org/ 10.3389/fpls.2021.650296.

BOSSOLANI, J. W.; CRUSCIOL, C. A. C.; MERLOTI, L. F.; MORETTI, L. G.; COSTA, N. R.; TSAI, S. M.; KURAMAE, E. E. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. **Geoderma**, n. 375, 114476, 2020. DOI: https://doi.org/10.1016/j.geoderma.2020.114476

CAIRES, E. F.; GARBUIO, F. J.; BARTH, G. Surface application of lime and cover black oat and corn residues for no-till soybean production. Communications in Soil **Science and Plant Analysis**, v. 39, p. 2102-2118, 2008. DOI: https://doi.org/10.1080/00103620802135138

CAIRES, E. F.; GUIMARÃES, A. M. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. **Agronomy Journal**, v. 110, n. 5, p. 1987-1995, 2018. DOI: https://doi.org/10.2134/agronj2017.11.0642

CAIRES, E. F.; HENRIQUE, E.; MASCHIETTO, G.; JOSÉ, F.; CHURKA, S.; ANTONIO, H.; JORIS, W. Surface application of gypsum in low acidic Oxisol under no-till cropping system. **Scientia Agrícola**, n. 2, p. 209-216, 2011.

CAIRES, E. F.; PEREIRA FILHO, P. R. S.; ZARDO FILHO, R.; FELDHAUS, I. C. Soil acidity and aluminium toxicity as affected by surface liming and cover oat residues under a no-till system. **Soil Use and Management**, v. 24, n. 3, p. 302-309, 2008.

CARMEIS FILHO, A. C. A.; CRUSCIOL, C. A. C.; CASTILHOS, A. M. Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. **Journal of Agricultural Science**, v. 155, n. 7, p. 1093-1112, 2017. DOI: https://doi.org/10.1017/S0021859617000235

CARMEIS FILHO, A. C. A.; CRUSCIOL, C. A. C.; GUIMARÃES, T. M.; CALONEGO, J. C.; MOONEY, S. J. Impact of amendments on the physical properties of soil under tropical long-term no till conditions. **PLoS One**, v. 12, n. 2, 27959897, 2017. DOI: https://doi.org/10.1371/journal.pone.0173011

COSTA, C. H. M.; CARMEIS FILHO, A. C. A.; CRUSCIOL, C. A. C.; SORATTO, R. P.; GUIMARÃES, T. M. Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. **Crop and Pasture Science**, v. 69, n. 5, 488-505, 2018. DOI: https://doi.org/10.1071/CP17233

CRUSCIOL, C. A. C.; MARQUES, R. R.; CARMEIS FILHO, A. C. A.; SORATTO, R. P.; COSTA, C. H. M.; FERRARI NETO, J.; CASTRO, G. S. A.; PARIZ, C. M.; CASTILHOS, A. M.; FRANZLUEBBERS, A. J. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. **Nutrient Cycling in Agroecosystems**, v. 115, p. 347-372, 2019. DOI: https://doi.org/10.1007/s10705-019-10017-0

CRUSCIOL, C. A. C.; MARQUES, R. R.; FILHO, A. C. A. C.; SORATTO, R. P.; COSTA, C. H. M.; NETO, J. F.; CASTRO, G. S. A.; PARIZ, C. M.; DE CASTILHOS, A. M. Annual crop rotation of tropical pastures with no-till soil as affected by lime surface application. **European Journal of Agronomy**, v. 80, p. 88-104, 2016. DOI: https://doi.org/10.1016/j.eja.2016.07.002

CUNNINGHAM, C. Characterization of dry spells in Southeastern Brazil during the monsoon season. **International Journal of Climatology**, v. 40, n. 10, p. 4609-4621, 2020. DOI: https://doi.org/10.1002/joc.6478

CUSSER, S.; BAHLAI, C.; SWINTON, S. M.; ROBERTSON, G. P.; HADDAD, N. M. Long-term research avoids spurious and misleading trends in sustainability attributes of no-till. **Global Change Biology**, v. 26, n. 6, p. 3715-3725, 2020. DOI: https://doi.org/10.1111/gcb.15080

DUVAL, M. E.; GALANTINI, J. A.; IGLESIAS, J. O.; CANELO, S.; MARTINEZ, J. M.; WALL, L. Analysis of organic fractions as indicators of soil quality under natural and cultivated systems. **Soil and Tillage Research**, v. 131, p. 11-19, 2013.

FAGERIA, N. K.; NASCENTE, A. S. Management of soil acidity of South American soils for sustainable crop production. **Advances in Agronomy**, v. 128, p. 221-275, 2014. DOI: http://dx.doi.org/10.1016/B978-0-12-802139-2.00006-8.

GROVER, S. P.; BUTTERLY, C. R.; WANG, X.; TANG, C. The short-term effects of liming on organic carbon mineralisation in two acidic soils as affected by different rates and application depths of lime. **Biology and Fertility of Soils**, v. 53, p. 431-443, 2017. DOI: https://doi.org/10.1007/s00374-017-1196-y

HOLLAND, J. E.; BENNETT, A. E.; NEWTON, A. C.; WHITE, P. J.; MCKENZIE, B. M.; GEORGE, T. S.; PAKEMAN, R. J.; BAILEY, J. S.; FORNARA, D. A.; HAYES, R. C. Liming impacts on soils, crops and biodiversity in the UK: A review. **Science of the Total Environment**, v. 610-611, p. 316–332, 2018. DOI: https://doi.org/10.1016/j.scitotenv.2017.08.020

INAGAKI, T. M.; DE MORAES SÁ, J. C.; CAIRES, E. F.; GONÇALVES, D. R. P. Lime and gypsum application increases biological activity, carbon pools, and agronomic productivity in highly weathered soil. **Agriculture, Ecosystems and Environment**, v. 231, p. 156-165, 2016. DOI: https://doi.org/10.1016/j.agee.2016.06.034

LI, Y.; CUI, S.; CHANG, S. X.; ZHANG, Q. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. **Journal of Soils and Sediments**, v. 19, n. 3, p. 1393–1406, 2019.

ROTH, C. H.; PAVAN, M. A. Effects of lime and gypsum on clay dispersion and infiltration in samples of a Brazilian Oxisol. **Geoderma**, v. 48, n. 3-4, p. 351-361, 1991. DOI: https://doi.org/10.1016/0016-7061(91)90053-V

SANTOS, D. R.; TIECHER, T.; GONZATTO, R.; SANTANNA, M. A.; BRUNETTO, G.; DA SILVA, L. S. 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 and Tillage Research**, v. 180, p. 222-231, 2018. DOI: https://doi.org/10.1016/j.still.2018.03.014

SORATTO, R. P.; CRUSCIOL, C. A. C. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. **Agronomy Journal**, v. 100, n. 2, p. 261-270, 2008. DOI: https://doi.org/10.2134/agronj2007.0120

SUMNER, M. E.; NOBLE, A. D. Soil acidification: the world story. *In:* RENGEL, Z. **Handbook of soil acidity**. New York: Marcel Dekker, 2003. p. 1-28.

TIRITAN, C. S.; BÜLL, L. T.; CRUSCIOL, C. A. C.; CARMEIS FILHO, A. C. A.; FERNANDES, D. M.; NASCENTE, A. S. Tillage system and lime application in a tropical region: Soil chemical fertility and corn yield in succession to degraded pastures. **Soil and Tillage Research**, v. 155, p. 437-447, 2016. DOI: https://doi.org/10.1016/j.still.2015.06.012

VAN RAIJ, B.; ANDRADE, J. C.; CANTARELLA, H.; QUAGGIO, J. A. **Análise química para avaliação da fertilidade de solos tropicais**. Campinas: Instituto Agronômico, 2001.

VITTI, G. C.; LUZ, P. H. C. **Utilização agronômica de corretivos agrícolas**. Piracicaba: FEALQ/GAPE, 2004.

VON UEXKÜLL, H. R.; MUTERT, E. Global extent, development and economic impact of acid soils. **Plant and Soil**, v. 171, p. 1-15, 1995. DOI: https://doi.org/10.1007/BF00009558