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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

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SOIL-PLANT SYSTEM**

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

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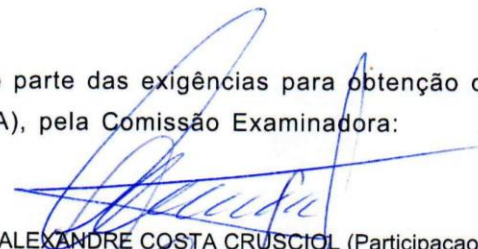
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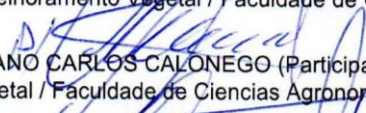
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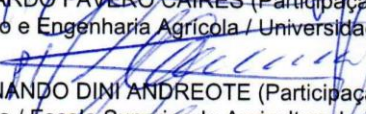
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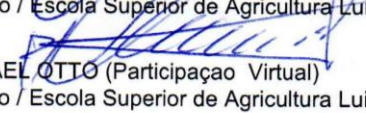
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“Aqueles que se sentem satisfeitos
sentam-se e nada fazem. Os insatisfeitos
são os únicos benfeitores do mundo”

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 [$(^{15}\text{NH}_4)_2\text{SO}_4$] 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; $(^{15}\text{NH}_4)_2\text{SO}_4$.

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 Agrônômicas 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, recuperação 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 [$(^{15}\text{NH}_4)_2\text{SO}_4$] 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, conseqüentemente, a produtividade de grãos das lavouras.

Palavras-chave: acidez do solo; aprofundamento radicular; microbiologia do solo; ciclo do nitrogênio; $(^{15}\text{NH}_4)_2\text{SO}_4$.

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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 mobility in the profile (low solubility in water) (SORATTO; CRUSCIOL, 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 ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). 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 strongly 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.

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 CO₂, 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 Ca²⁺ and Mg²⁺ 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.

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