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JÉSSICA PIGATTO DE QUEIROZ BARCELOS

DINÂMICA DO NITROGÊNIO E CARBONO EM UM SISTEMA DE PRODUÇÃO EM FUNÇÃO DA CALAGEM, GESSAGEM E ADUBAÇÃO NITROGENADA

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Campus de Botucatu, para obtenção do título de Doutora em Agronomia (Agricultura)

Orientador: Prof. Dr. Ciro Antonio Rosolem

Coorientador: Prof. Dr. Thiago A.R. Nogueira

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EMBRAPA SOJA

Botucatu, 01 de junho de 2021

Aos meus pais Selma e Valter,

Ao meu irmão Gabriel,

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RESUMO

Altas produtividades das culturas somente são possíveis diante da correção da acidez dos solos e neutralização do alumínio toxico. O calcário aplicado superficialmente tem seu efeito de forma lenta pelo perfil do solo, de forma que a associação com gesso se torna interessante ao aumentar a fertilidade em subsuperfície, favorecendo o crescimento radicular, aumentando o acúmulo de C em profundidade. A dinâmica do nitrogênio (N) e do carbono (C) no sistema solo-planta-atmosfera e a atividade microbiana do solo podem ser influenciadas pela aplicação superficial do calcário e do gesso e pela mudança do pH. Para avaliar a interação entre a aplicação de calcário, gesso e fertilizante nitrogenado nos balanços de N e C, foi realizado um experimento, em sistema de plantio direto com a sucessão de soja – milho safrinha, com milho consorciado com capim Tânzania (*Megathyrsus maximus* cv Tanzânia) que foi deixado no campo como planta de cobertura. Calcário e gesso foram aplicados antes da semeadura da cultura da soja em outubro de 2016, e novamente em outubro de 2017. Duas doses de N (0 e 160 kg ha^{-1} de N, sulfato de amônio) foram aplicadas anualmente na cultura do milho. Foram determinadas as entradas e saídas de N e C, e os balanços parciais de N e C. Baseado nos resultados obtidos no experimento de campo, foram realizadas incubações em laboratório com solos de cada tratamento em duas profundidades (0.0-0.10 m e 0.40-0.60 m). Foram adicionados ao solo substratos marcados com ^{14}C em baixas concentrações para determinar a atividade microbiana, a eficiência de uso do C, e a mineralização de C e N do solo. Foi avaliado o perfil da comunidade microbiana do solo de amostras da camada arável, utilizando as metodologias phospholipid fatty acid (PLFA) e sequenciamento Illumina 16S ribosomal RNA (rRNA) amplicon. Embora o balanço parcial de N tenha apresentado resultado negativo, tanto o incremento anual de N no solo foi positivo, assim como o incremento anual de C. Os tratamentos com calcário apresentaram maior potencial de mineralização, e a aplicação de calcário e fertilizante nitrogenado apresentou maior nitrificação no solo. Apesar das diferenças entre a camada arável e o subsolo, e das diferenças nas características do solo causadas pela aplicação de N, a aplicação de calcário e gesso não resulta em diferença na eficiência de uso do ^{14}C-glicose. Entretanto, os resultados não estavam diretamente relacionados ao aumento de pH ocasionado pela aplicação de calcário. Apesar disso, ao aliviar o estresse por acidez do solo, a aplicação de corretivos favoreceu a abundância de grupos microbianos considerados benéficos, como de fungos micorrízicos arbusculares e gêneros de bactérias envolvidas no ciclo do N. Além disso, apesar de não ocorrerem mudanças a nível de Filo, em curto período, as mudanças observadas auxiliam a predizer futuros comportamentos da comunidade microbiana.

Palavras-chave: *Glycine max* L.; *Zea mays* L.; adubação nitrogenada; gases de efeito estufa; manejo conservacionista; reação do solo.

ABSTRACT

High crop yields are only possible when correcting soil acidity and neutralizing toxic aluminium. Lime when applied superficially moves very slowly in the soil profile, hence its association with gypsum is interesting to ameliorate sub-soil fertility favouring root growth, increasing the accumulation of C in depth. The dynamics of nitrogen (N) and carbon (C) in the soil-plant-atmosphere system and the microbial activity of the soil can be influenced by application of lime and gypsum, and consequently change in the soil pH. To evaluate the interaction between the application of lime, gypsum and nitrogen fertilizer in the balances of N and C, an experiment was carried out, in a no-tillage system with soybean followed by maize-off season, with maize intercropped with guinea grass (*Megathyrsus maximus* cv Tanzania) as a relay crop. Lime and gypsum were applied before soybean planting in October 2016, and again in October 2017. Two N rates (0, 160 kg ha^{-1} of N as ammonium sulphate) were applied annually to maize. N and C inputs, outputs and partial N and C balances were determined. Based on the results obtained in the field experiment, incubations were carried out in laboratories with soils from each treatment at two depths (0.0-0.10 m and 0.40-0.60 m). ^{14}C-labelled substrates were added at low concentrations to determinate microbial activity, microbial substrate use efficiency, and C and N mineralization in the soil. We evaluated the soil microbial community profile from topsoil samples, using phospholipid fatty acid (PLFA) and Illumina 16S ribosomal RNA (rRNA) amplicon sequencing. Although the partial balance of N has shown a negative result, both annual increments of C and N in the soil were positive. The treatments with lime showed greater potential for mineralization, and the application of lime and N-fertilizer. presented higher nitrification in the soil. Despite the differences between the topsoil and the subsoil, and the differences in soil characteristics caused by the application of N, the application of lime and gypsum did not result in a difference in the ^{14}C-glucose use efficiency. Thus, the observed results were not directly related to the pH increase caused by the application of limestone. However, soil amendments favour the abundance of beneficial microbial groups such as arbuscular mycorrhizal fungi and bacteria genera involved in N cycle, by alleviating soil acidity stress. Moreover, despite no changes found in the phylum-level in the short term, it assists with the capacity to predict the future behaviours of soil microbial communities.

Keywords: *Glycine max* L.; *Zea mays* L.; nitrogen fertilization; greenhouse gases emission; conservative management; soil acidity.

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SUMMARY

GENERAL INTRODUCTION

Soil comprises one of the largest reserves of carbon (C) in the biosphere (RIGON; CALONEGO, 2020), storing two thirds of the total terrestrial C (LORENZ; LAL, 2018). However, depending on the management, the soil can act as net sink of C or a net source, emitting greenhouse gases responsible for the global warming. Increasing soil organic C in deeper layers has been considered a strategy to enhance sequestration (LORENZ; LAL, 2018). In addition, recent research indicates that the implementation of conservation agriculture managements such as the no-till system, maintaining soil cover, associated with crop rotation and diversification of plant species (FAO, 2017), increase organic carbon sequestration and mitigation of CO_2 emissions to the atmosphere (RIGON et al., 2021; RIGON; CALONEGO, 2020). A common agricultural practice in Brazil involves growing soybean [*Glycine max* (L.) Merrill] followed by maize (*Zea mays* L.) in the off season. Thus, plants such as tropical perennial grasses are generally cultivated in no-till systems as cover crops, because of its prolonged persistence in the soil surface and their higher C:N ratio (GRASSMANN et al., 2020; ROSOLEM et al., 2017). Thus, to optimize the cropping system the off-season-maize can be grown intercropped with grass species such as Guinea grass (*Megathyrsus maximum* cv. Tanzânia) improving plant biomass production, soil covering and subsoil root exploration (BOSSOLANI et al., 2020; ROCHA et al., 2020).

When subjected to intensive agricultural systems, if not properly managed, soils can be susceptible to N depletion through nutrient removal (RAPHAEL et al., 2016; RIGON; CALONEGO, 2020; ROCHA et al., 2020), leading to soil organic matter and C loss through erosion and leaching process, resulting in soil degradation (PURWANTO; ALAM, 2020; RAPHAEL et al., 2016). In general, the inclusion of leguminous species helps to reduce the dependence on N mineral fertilizer in the system (LÖTJÖNEN; OLLIKAINEN, 2017). Soybean symbioses can supply the amount of N required to reach high yield, and still release N from plant residues into the soil (HOLLAND et al., 2018; HUNGRIA; MENDES, 2015). However, to achieve high-yield levels in maize, and avoid competition with the intercropping grass and sustain the system, N fertilizer supply may be critical (MATEUS et al., 2020; ROCHA et al., 2020). The addition of ammonium–based N fertilizer and the biological nitrogen fixation (BNF) process (AHMAD et al., 2020; NYATSANGA; PIERRE, 1973), altogether with the removal of cations from the soil during intensive agriculture and natural processes (via C, N and S cycling) may increase soil acidity (HOLLAND et al., 2018; KUNHIKRISHNAN et al., 2016).

Soil acidity is one of the primary factors limiting crop development and productive potential, mainly in tropical areas (BOSSOLANI et al., 2020; HOLLAND et al., 2018; RITCHEY et al., 1980). Low soil pH, cause adverse effects on plants and soil microorganisms, reducing nutrient availability, and restricting plant root development and access to water and nutrient (KUNHIKRISHNAN et al., 2016; RITCHEY et al., 1980). Liming is most common practice to alleviate soil acidity, but due to its low solubility and to the surface application in no-till systems, the reaction may be restricted to the first soil layers (CARMEIS FILHO et al., 2017; FERRARI NETO et al., 2021; RITCHEY et al., 1980). Gypsum ($CaSO_4{\cdot}H_2O$) generally does not affect soil pH, but present higher solubility compared with lime, promoting the movement of sulphate (SO_4^{2-}) through the soil profile carrying cations as Ca^{2+} and reducing Al^{3+} activity, providing a better environment for root growth (CARMEIS FILHO et al., 2017; RITCHEY et al., 1980; VIEIRA FONTOURA et al., 2019). Then, especially in the short-term gypsum can be recommended as a soil conditioner (HOLLAND et al., 2018; RITCHEY et al., 1980; ZOCA; PENN, 2017). Benefits resulting from the combination of lime and gypsum can include increase subsoil fertility and root growth deep down in soil profile, greater plant biomass production and higher crop yield, increasing C inputs in the soil (CARMEIS FILHO et al., 2017; DA COSTA; CRUSCIOL, 2016; FERRARI NETO et al., 2021; INAGAKI et al., 2017).

In addition, soil pH is tightly associated with shifts in soil microbial community structure (JONES et al., 2019). Low soil pH critically affects bacteria diversity (ZHALNINA et al., 2015), fungi:bacteria ratios (ROUSK; BROOKES; BÅÅTH, 2010), N mineralization (ACIEGO PIETRI; BROOKES, 2008), microbial C use efficiency (CUE) and turnover rates (JONES et al., 2019; MALIK et al., 2018), among others. Furthermore, microbial trade-off to soil acidity stress adaptation and biomass production lead to lower potential for C storage through increased microbial growth efficiency (MALIK et al., 2018). Thus, liming associated with gypsum can have a positive effect on microbial biomass and microbial activity (GARBUIO et al., 2011; INAGAKI et al., 2017; KEMMITT et al., 2006), with subsequent effects on C mineralization and N mineralization throughout the soil profile (GARBUIO et al., 2011; WACHENDORF, 2015). Liming is frequently associated with increase in carbon dioxide (CO_2) emission during it chemical dissolution by increasing soil biological activity and hence soil respiration (ABALOS et al., 2020; GIBBONS et al., 2014; KUNHIKRISHNAN et al., 2016; WACHENDORF, 2015). Lime and gypsum addition, by decreasing Al^{3+} ions availability, may favour methane (CH_4) oxidizers activity, increasing CH_4 uptake (ABALOS et al., 2020; LINDAU et al., 1994). Repeated applications of lime are likely to increase soil N mineralization, but the occurrence of net mineralization or net immobilization resulting from liming will depend on the C:N ratio and the quality of the organic matter (HOLLAND et al., 2018; WACHENDORF, 2015). In general, liming is associate with decrease in nitrous oxide (N_2O) emission (ABALOS et al., 2020; HOLLAND et al., 2018; KUNHIKRISHNAN et al., 2016), and increases in ammonia (NH_3) volatilization (SMITH et al., 2009; SOMMER; ERSBØLL, 1996) and nitrate (NO_3^-) leaching (GIBBONS et al., 2014; KEMMITT et al., 2006; ROSOLEM et al., 2017). Therefore, understanding this complex dynamic in addition to the effects N fertilizer addition may be important for the mitigation of climate change, seeking the best management to increase C sequestration and avoid C and N losses in the system.

In the present study we discuss how liming, and the co-addition of gypsum and N fertilizer affect the mechanisms and dynamics of C and N in the system, and its importance for the mitigation of climate change. The positive increments of C and N in the system, in addition to the positive/unexpected effects of treatments on accumulated CO_2-C emissions, pointed out in the first chapter were fundamental for stimulate a search for detailed explanations of how the treatments were affecting soil microbial activity and microbial community profile. The results presented here reveal new perspectives on how the management of lime and gypsum affects the dynamics of C and N inserted in a production system in a short-term through a set of complementary methods, allowing to achieve a holistic understanding of the C and N cycle, and how lime and gypsum may reflect on some of the drivers of climate change.

FINAL CONSIDERATIONS

Soil acidity is one of the most important limitations to crop production in the world. Although the primary aim of liming being overcoming soil acidity, it impacts the chemical, biological and physical properties of soils, thereby affecting microbial activity, transformations of C and N in soils and GHG fluxes. Despite the well-known benefits of lime application on crop production, there are some drawbacks and negative impacts of liming in the net climate change. As the CO_2 emissions produced during neutralization of acidity, or possible effect on in nitrate leaching due to stimulation of lime in mineralization and nitrification process have been discussed. During the incubation test, treatments with lime and gypsum have resulted in greater $^{14}CO_2$ respired, irrespective of N fertilizer management or soil depth. However, the CUE showed no difference between treatments, in accordance with the accumulated CO_2-C emissions observed under field condition. In addition, the CUE of ^{14}C-glucose was similar between soil layers, demonstrating potential for C sequestration. Throughout the experiment it became clear that the use of strategies such as application of gypsum and nitrogen fertilizer, as well as the system of cultivation with legumes in combination with grasses, favoured the cycling of N and increased the fixation of C. The cultivation of soybean in fact reduced the dependence on N fertilizer in the system, while intercropping maize and guinea grass favoured N cycle, since compared with the literature it was observed general low $NO3^-$-N leaching and great plant biomass production. In addition, despite the partial negative N balance, the application of lime promoted an increase of C and N in the systems, in addition to greater grain production. It was demonstrated that the results of CUE and the mineralization of C and N are not directly related to the change in pH caused by the application of lime. The microbial community profile revealed that the abundance of beneficial microbial groups, such as AM fungi and N cycle bacteria, as favoured by alleviating soil acidity stress with the addition of lime and gypsum. Among the changes obtained by the soil amendments, increasing in Fungi:Bacteria ratio, and decreasing in Gram+:Gram- ratios are suggested to be a microbial community with better CUE. Thus, these earliest founds indicates that even in short-term changes in soil pH is a critical driver of change for the microbial community composition, and the alleviation on acidity is beneficial to C and N cycles.

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