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INTERAÇÃO DA CORREÇÃO DA ACIDEZ DO SOLO COM NITROGÊNIO NO ACÚMULO DE CARBONO ESTÁVEL NO SOLO

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Advisor: Prof. Dr. Ciro Antonio Rosolem

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To my beloved parents Waldirene and Eduardo,
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ABSTRACT

The effect of the direct seeding system on the accumulation of carbon (C) in the soil is well known. The amount of labile C in the soil is related to the quantity and quality of straw in the system. However, the accumulation of stable C, linked to soil minerals, is more interesting due to its greater resilience. It is known that conservation production systems improve soil aggregation and that smaller aggregates accumulate stable C, which, in turn, depends on the nitrogen (N) present in the system and the presence of cationic bridges. Although the effect of each of these factors is known, little is known about the interaction between them. The objectivewas to study the effect of applying limestone and agricultural gypsum, associated or not with nitrogen fertilization, on soil aggregation, accumulation of C and humic substances, relating theaccumulation of stable C with the classes of aggregates in a production system with soybeans and corn intercropped with Tanzania grass, in the second harvest. The field experiment was installed in Botucatu-SP, in 2016, and the data presented are from 2020 to 2022. The treatmentswere: control (without application of limestone and gypsum), limestone, and limestone + gypsum, with combined doses of N of 0, 80, 160, and 240 kg ha-1, applied annually to corn. Soybeans were grown in the spring/summer and corn in the fall/winter of each year. The combination of limestone, gypsum, and nitrogen fertilizer led to a 13% higher total C stock anda 20% higher total N stock in the 0-60 cm soil profile compared to the control. Liming + N reduced fulvic acids (FA) and humic acids (HA), but the application of gypsum mitigated this negative effect. The application of lime, gypsum, and N increased humin levels, mainly on the soil surface. Thus, the best strategy to increase C storage and stabilization in humid tropical soils in double soybean-corn cultivation was the combined use of limestone, gypsum, and nitrogen fertilizer applied to corn, achieving greater C storage in larger aggregates. The application of limestone and gypsum resulted in lower carbon mineralization compared to the control under N deficiency. N mineralization was greater when limestone + gypsum was appliedtogether with N. Liming and nitrogen fertilization significantly increased nitrate levels in the soil, with no differences between liming and liming + plaster. Greater microbial activity, carbon mineralization, and nitrification were observed when N was applied to the

soil. Furthermore, it was observed that the application of liming and liming + gypsum played a substantial role in the processes involving C mineralization in soils, with greater effects on smaller aggregates. Liming management and gypsum application can extend these benefits to deeper soil layers, resulting in increased soil fertility in subsurface layers. Soil management using limestone + gypsum proved to be a promising practice in increasing the productivity of soybeans and corn. Grains, especially at a lower dose of N (80 kg ha-1) or in the absence of nitrogen fertilizer. This result demonstrates the positive impact of liming and gypsum management on the N-use efficiency of crops, thus reducing dependence on nitrogen fertilization in acidic soils. Productivity gains were directly linked to the physical (greater stability and diameter of aggregates) and chemical (pH and macronutrient content) improvements in the soil provided by limestone + gypsum management. Therefore, it is concluded that the synergism of soil correction practices with liming, gypsum, and nitrogen fertilization applied to second-crop corn intercropped with forage species resulted in greater grain productivity, increased soil fertility, C sequestration, and storage, especially in larger aggregates.

Keywords: nitrogen fertilization; soil aggregates; liming; *Glycine max* L; *Zea mays* L.

RESUMO

É bem conhecido o efeito do sistema de semeadura direta no acúmulo de carbono (C) no solo. A quantidade de C lábil no solo tem relação com a quantidade e qualidade de palha do sistema. Entretanto, o acúmulo de C estável, ligado aos minerais do solo, é mais interessante devido à sua maior resiliência. Sabe-se que sistemas de produção conservacionistas melhoram a agregação do solo, e que agregados menores acumulam C estável, o que, por sua vez, depende do nitrogênio (N) presente no sistema e da presença de pontes catiônicas. Embora o efeito de cada um desses fatores seja conhecido, pouco se sabe sobre a interação entre eles. Objetivou- se estudar o efeito da aplicação de calcário e gesso agrícola, associados ou não à adubação nitrogenada, na agregação do solo, acúmulo de C e substâncias húmicas, relacionando o acúmulo de C estável com as classes de agregados em um sistema de produção com soja e milho consorciado com capim Tanzania, em segunda safra. O experimento de campo foi instalado em Botucatu-SP, no ano de 2016, e os dados apresentados são de 2020 a 2022. Os tratamentos foram: controle (sem aplicação de calcário e gesso), calcário, e calcário + gesso, com doses combinadas de N de 0, 80, 160, 240 kg ha^{-1}, aplicadas anualmente ao milho. A soja foi cultivada na primavera/verão e o milho no outono/inverno de cada ano. A combinação de calcário, gesso e adubação nitrogenada levou a um estoque total de C 13% maior e um estoque total de N 20% maior no perfil de solo de 0-60 cm em comparação com o controle. A calagem + N reduziu ácidos fúlvicos (AF) e ácidos húmicos (AH), mas a aplicação de gesso mitigou esse efeito negativo. A aplicação de calcário, gesso e N aumentou os teores de humina, principalmente na superfície do solo. Assim, a melhor estratégia para aumentar o armazenamento e estabilização de C em solos tropicais úmidos no duplo cultivo soja-milho foi o uso combinado de calcário, gesso e adubação nitrogenada aplicada ao milho, alcançando maior armazenamento de C em agregados maiores. A aplicação de calcário e gesso resultou em menor mineralização de carbono comparado ao controle sob deficiência de N. A mineralização de N foi maior quando calcário + gesso foi aplicado junto com N. A calagem e a adubação nitrogenada aumentaram significativamente os níveis de nitrato no solo, sem diferenças entre calagem e calagem + gesso. Foi observada maior atividade microbiana, mineralização de carbono e nitrificação quando N é

aplicado no solo. Além disso, observou-se que a aplicação de calagem e calagem + gesso desempenhou um papel substancial nos processos que envolvem a mineralização de C nos solos, com maiores efeitos em agregados menores. O manejo de calagem e aplicação de gesso pode estender esses benefícios para camadas mais profundas do solo, resultando em um aumento da fertilidade do solo nas camadas subsuperficiais. O manejo do solo utilizando calcário + gesso mostrou-se uma prática promissora no aumento da produtividade de grãos de soja e milho, especialmente na menor dose de N (80 kg ha^{-1}) ou na ausência da adubação nitrogenada. Este resultado demonstra o impacto positivo do manejo da calagem e gessagem na eficiência de uso de N das culturas, reduzindo assim a dependência da adubação nitrogenada em solos ácidos. Os ganhos de produtividade foram diretamente ligados à melhoria física (maior estabilidade e diâmetro de agregados) e química (pH e teores de macronutrientes) do solo proporcionadas pelo manejo de calcário + gesso. Portanto, conclui-se que o sinergismo das práticas de correção do solo com calagem, gessagem e adubação nitrogenada aplicadas no milho segunda safra consorciado com espécie forrageira resultou em maior produtividade de grãos, incremento da fertilidade do solo, sequestro e armazenamento de C, especialmente em agregados de maior tamanho.

Palavras-chave: adubação nitrogenada; agregados de solo; calagem; *Glycine max* L; *Zea mays* L.

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GENERAL INTRODUCTION

Soil quality is directly related to carbon (C) content and is influenced by management practices such as crop rotation and no-till farming systems. A crucial factor for the accumulation of organic carbon in soil is the availability of nitrogen (N) (Boddey *et al*., 2010), as well as the increase in microbial populations in the rhizosphere (Cheng *et al*., 2008). Grass residues with a high C/N ratio persist longer in soil due to their low decomposition rate (Teixeira *et al*., 2014), which favors the accumulation of soil organic matter (SOM) and reduces mineralization (Shahbaz *et al*., 2017). Thus, crop rotation and the type of farming system influence the accumulation of organic carbon, biological activity, and soil aggregation. Carbon protection within stable aggregates inhibits mineralization and increases C retention in soil (Cambardella and Elliott, 1992). This carbon can be trapped in aggregates and physically protected from microbial activity (Tisdall and Oades, 1982), or adsorbed onto mineral surfaces (Janzen, 2015).

Increasing carbon stocks in tropical soils in humid regions is a challenge, especially in more stable fractions such as mineral-associated C and humic substances (Cordeiro *et al*., 2022), since although susceptible to mineralization, soil organic carbon can be protected by the mineral matrix (Kögel-Knabner and Amelung, 2014). Aggregates formed by the trapping carbon not only physically protect organic matter (Tisdall and Oades, 1982) but also influence microbial community structure (Hattori, 1988), limiting oxygen diffusion (Sexstone *et al*., 1985), regulating water flow (Prove *et al*., 1990), determining nutrient adsorption and desorption (Wang et al., 2001), and reducing surface runoff and erosion (Barthe and Roose, 2002).

In this sense, improving soil aggregation is crucial for increasing organic matter physical protection and C stability (Rowley *et al*., 2018). The application of lime and agricultural gypsum improves soil aggregation, as calcium (Ca) and magnesium (Mg) act as flocculating agents that stabilize C (Castro *et al*., 2015; Rowley *et al*., 2018). Gypsum also protects C by providing available Ca, improving aggregation with clay and silt, and promoting the growth of fine roots, which are sources of recalcitrant compounds (Carvalho *et al*., 2023). Additionally, gypsum is an excellent source of sulfur, essential for biological nitrogen fixation and soybean

productivity (Almeida *et al*., 2023; Virk *et al*., 2022). The application of gypsum alone has shown limited benefits for C capture (Walia *et al*., 2023). Low soil fertility, especially nitrogen, phosphorus, and Ca deficiencies, can restrict C capture (Mattila *et al*., 2022). The main impact of lime and gypsum on C capture is related to increased biomass production, especially roots (Costa *et al*., 2021), promoting greater C stocks at depth (Araujo *et al*., 2019).

In addition, nitrogen fertilization can enhance root growth, facilitating the movement of Ca supplied by lime (Foloni *et al*., 2006; Crusciol *et al*., 2022). Increased C capture is associated with increased crop productivity, especially in C4 plants, achieved through soil correction and integrated production systems (Siddique *et al*., 2024). In production systems where soybean and maize are intercropped with forage grasses soil aggregation and C and N contents can increase (Cooper *et al*., 2021; Barcelos *et al*., 2022). However, in these systems, lime is applied to the soil surface, and due to the low solubility and mobility of Ca, it is common to apply gypsum together with lime to alleviate aluminum toxicity at depth and improve root growth (Pivetta *et al*., 2019). In these systems, N is not applied to soybeans due to the high efficiency of biological nitrogen fixation (BNF), and N fertilization in maize is usually low, which may result in a negative N balance (Rocha *et al*., 2020; Barcelos *et al*., 2022).

Nitrogen deficiency and soil acidity can limit C storage in stable fractions. Humic substances, such as humic acid (HA), fulvic acid (FA), and humin, are stable components of organic matter, with stability in decreasing order (Tadini *et al*., 2022). Mitigating soil acidity with lime increases microbial activity and soil respiration, which can result in some C loss. However, in systems with cover crops, this has not been reported (Cordeiro *et al*., 2021). Moreover, the amount of C loss is insignificant compared to the higher dry matter production and addition of cations that stabilize soil C (Barcelos *et al*., 2022). The net balance is a greater efficiency of C use by the soil microbiota (Barcelos *et al*., 2021). Thus, the capture and humification of soil C may increase with improved soil fertility (Ovchinnikova, 2018). The lime application increases humic substance levels in the soil's surface layer (Mockeviciene *et al*., 2022), but few studies have examined the combined use of lime and gypsum at depth, especially in no-till systems. In conservation systems with cover crops, humic C is stored mainly in macroaggregates through the physical interlocking of microaggregates with roots. Newly formed and more

labile HAs are found in greater quantities in macroaggregates, while microaggregates contain more recalcitrant HAs. However, these effects were only found near the soil surface (Ndzelu *et al*., 2021), and it is unknown whether the application of lime and gypsum associated with N fertilization can affect the distribution of humic substances in deeper soil layers. Both N and lime initially accelerate SOM mineralization due to increased microbial activity, however, the more stable soil C fractions are enhanced in conservation systems through C physical protection within aggregates (Neff *et al*., 2002; Castro *et al*., 2015). Nitrogen fertilization affects the dynamics of soil humic substances (Kou *et al*., 2022), although this has not been described in tropical climates.

Crop residue accumulation on the soil surface provides inputs of C and N and promotes a zone of higher potential for soil biological activity, measured by C and N mineralization under laboratory conditions (Franzluebbers *et al*., 2018). Soil organic carbon is directly associated with total nitrogen, with strong biological links (Cleveland and Liptzin, 2007), indicating that any change in organic carbon will also affect total soil N, dominated by the organic fraction (Pringle *et al.,* 2014). N fertilization accelerates the decomposition of light carbon fractions while stabilizing carbon compounds in heavy fractions in soils, as well as it has been shown to increase C in the rhizosphere due to higher rhizodeposition and aggregate formation (Chu *et al*., 2007). In addition, when nitrogen is associated with more persistent minerals it is less susceptible to microbial mineralization and subsequent leaching and gaseous losses (Kelley and Stevenson, 1995). In soils cultivated with grasses, which provide lignin-rich residues, N fertilizer application increases carbon sequestration (Jones *et al*., 2009), reducing microbial lignin's activity (Fog, 1988).

Given the above, to confirm the hypothesis that liming associated with the addition of agricultural gypsum and N in a no-till production system can result in a greater accumulation of stable C in soils than each soil amendment alone, this study was conducted to verify (i) accumulation of C, total N, and humic substances in different soil aggregate classes (ii) aggregation and soil fertility in deeper no-till production system soil layers and increased crop yield (iii) C stability, C mineralization potential, and microbial biomass C in a no-till production system.

CHAPTER 1

SOIL AMENDMENT AND N FERTILIZATION STRATEGIES TO IMPROVE C SEQUESTRATION AND STORAGE IN SOIL AGGREGATES[1]

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1.1 Introduction

One of the main challenges in tropical soils of humid regions is to maintain or increasecarbon (C) stock, especially in fractions of greater stability, such as C associated with mineralsand humic substances (Cordeiro et al., 2022). Improving soil aggregation is one of the main strategies to increase physical protection and C stability (Rowley et al., 2018). Liming and gypsum application can improve soil aggregation because calcium (Ca) and magnesium (Mg) are flocculating agents favoring C stabilization when applied at appropriate rates (Castro et al.,2015, Rowley et al., 2018).

Furthermore, it has recently been reported that gypsum physically protects C, providing accessible Ca, improving C aggregation with clay and silt, and enhancing the growthof fine plant roots that are a source of recalcitrant compounds, which have an important role inC stabilization (Carvalho et al., 2023). Additionally, gypsum is an excellent source of sulfur, which is important because sulfur deficiency limits biological N fixation and soybean yield (Almeida et al., 2023), thereby potentially altering C sequestration (Virk et al., 2022). However,gypsum alone appears to have few benefits for C sequestration (Walia et al., 2023). Therefore, low soil fertility, mainly of N, phosphorus, and Ca, can limit C sequestration (Mattila et al., 2022).

The primary benefit of liming and gypsum on C sequestration has been associated withhigher biomass production, mainly that of root production (Costa et al., 2021). For instance, gypsum was shown to improve soil C stock with depth due to greater root growth in the soil profile (Araujo et al., 2019). Furthermore, N fertilization can improve root growth in the soil profile by facilitating the downward movement of Ca supplied with limestone (Foloni et al., 2006, Crusciol et al., 2022). Therefore, a key point for increasing C sequestration should be associated with greater crop yield, and this can be achieved with soil correction and integrated production systems (Siddique et al., 2024).

[1] Chapter written following the guidelines of the Soil Science Society of America Journal

Integrated production systems with soybean [*Glycine max* (L.) Merrill] double-cropped with maize (*Zea mays* L.) intercropped with forage grasses have been reported to increase soil aggregation and soil C and N contents (Cooper, et al., 2021, Barcelos et al., 2022).However, crops are grown under no-till in these systems, and lime is applied on the soil surface.Since the solubility and mobility of Ca from lime are low, it is usual to apply gypsum together with lime to alleviate aluminum toxicity at depth and allow better root growth (Pivetta et al., 2019). In this production system, N is not applied to soybeans due to the high efficiency of biological N fixation, and N fertilization to maize is usually low, which can result in a negativeN balance (Rocha et al., 2020, Barcelos et al., 2022). This might impair soil C stabilization, since the C: N ratio of the humified soil organic matter remains relatively constant (e.g. 10) andthe N deficit could impair the stabilization of organic matter (Stevenson, 1994). Therefore, N deficiency and soil acidity might limit soil C storage in stable fractions.

Humic acid (HA), fulvic acid (FA), and humin (H) are stable components of organic matter, with stability levels following the order: HA > FA > H (Tadini et al., 2022). Soil pH alleviation by liming increases microbial activity and soil respiration, which could result in some C loss, but in systems with cover crops this has not been reported (Cordeiro et al., 2021a).Additionally, when existent, the amount of C lost is negligible given the greater dry matter production and addition of cations that stabilize soil C (Barcelos et al., 2022). The net balance is a greater efficiency of C use by soil microbiota (Barcelos et al., 2021). Thus, sequestration and humification of soil C can increase with improved soil fertility (Ovchinnikova, 2018). It has been observed that liming increases the contents of humic substances in the soil surface layer (Mockeviciene et al., 2022), but there are still few studies on the combined use of lime and gypsum on humic substances with soil depth, especially in systems under no-till, where lime and gypsum are not plowed into the soil. In conservation systems with the use of cover crops, C in the humic fraction is stored mainly in macroaggregates through physical enmeshment of microaggregates with roots. Newly formed and more labile HA are found in greater quantity in macroagregates, while microagregates contain more recalcitrant HA. However, these effects were found only in soil near the surface (Ndzelu et al., 2021), and it is not known so far whether lime and gypsum application associated with N fertilization can affectthe distribution of humic substances among soil aggregate classes with depth.

Both N and liming initially accelerate soil organic matter mineralization, mainly

of light fractions of C, due to greater soil microbial activity. Nevertheless, the most stable fractionsof soil C, especially C associated with minerals are enhanced in conservation systems through physical protection within the aggregates (Neff et al., 2002, Castro et al., 2015). It is also known that N fertilization affects the dynamics of soil humic substances (Kou et al., 2022), although this has not been described under tropical climates.

We hypothesized that the combination of lime, phosphogypsum, and N fertilization would result in greater soil C, N, and humic substances than if each of these amendments were applied independently. Moreover, we hypothesized that the accumulation would be differentiated by soil aggregate size class. The objective of this study was to evaluate the effectof limestone, phosphogypsum, and N fertilization on the accumulation of C, total N, and humicsubstances in different soil aggregate classes in a cropping system with double-cropped soybeanand maize intercropped with Guinea grass (*Megathyrsus maximum*, cv Tanzania).

1.2 Material and Methods

1.2.1 Characterization of the study área

A no-till field experiment was conducted in Botucatu, State of São Paulo, Brazil (22° 49' 51.6" S; 48° 25' 38.6" W). The climate is mesothermal with wet summers and dry winters. The average annual temperature is 20.7 °C, with a monthly average maximum of 26.1º C and a minimum of 15.3 °C. The annual average rainfall is 1696 mm. The soil is classified as Typic Haplorthox (Soil Survey Staff, 2014), corresponding to Oxisols (Jahn et al., 2006). The soil fertility attributes before the implementation of the study are in Table 1. The experiment was started in October 2016 to evaluate the response of soybean double-cropped with maize intercropped with Guinea grass to liming, phosphogypsum, and N fertilization.

Table 1 - Soil chemical and physical attributes and particle size distribution before the implementation of the experiment in August 2016.

Layer	pH	OC	P_{resin}	S	Al	K	Ca	Mg	CEC	Sand	Clay
	$CaCl_2$	g kg^{-1}	mg kg^{-1}			mg kg^{-1}			$mmol_c$ kg^{-1}	g kg^{-1}	
0-10 cm	4.3	10.2	12.8	4.8	84	81.9	238	141	78.2	540	343
10-20 cm	4.2	8.1	10.1	4.7	81	42.9	188	90	65.6	520	368
20-40 cm	4.1	7.1	11.4	4.8	111	19.5	74	49	63.1	518	410
40-60 cm	3.9	6.3	5.9	10.8	180	15.6	68	42	97.0	510	420

OC = organic carbon; Presin: Phosphorus extracted by ion exchange resin; CEC = cation exchange capacity.

1.2.2 Experimental planning and treatments

The experimental design was a factorial 3x2 design with the six treatments randomizedin four blocks. Treatments consisted of a control, lime, and lime + gypsum factorially arrangedwith two N rates. The control received no lime or gypsum. Lime was applied at 2.9 Mg ha^{-1} and gypsum at 2.0 Mg ha^{-1} in 2016, 2018, and 2020, before soybean planting. Nitrogen was applied annually to corn intercropped with Guinea grass cv. "Tanzania" according to farmers' practices, at rates of 0 and 240 kg ha^{-1} in the form of ammonium sulfate. The lime rate was initially calculated to raise the soil base saturation to 60% in the soil surface layer (0 -20 cm), and the gypsum rate was calculated by multiplying the clay content in the 20-40 cm soil layer by 6 (Quaggio & Van Raij, 1997). The N fertilizer was split as 30 kg ha^{-1} at planting and the remainder broadcast at maize stage V7/V8. Soybean was always planted in spring/summer (October-November) and maize + guinea grass as an intercropping system was planted in the fall (March). All plots were 4.5 m wide and 10.0 m long.

1.2.3 Soil Sampling and Aggregate Separation

In October 2020, monoliths 15 x 20 cm horizontally and 10 cm vertically were collected at 0-10, 10-20, 20-40, and 40-60 cm depths per plot. Three samples randomly taken from each plot were combined into a composite sample. Samples were placed in

plastic bags, protected from light, and transported to the laboratory.

Monoliths were gently fragmented into smaller fractions by passing through an 8-mm

- sieve but retained on a sieve with 4 mm openings. Soil retained on the 4 mm sieve was driedat 50 °C for 48 hours in a forced air oven. Water-stable aggregate classes were further separatedusing wet sieving (Yoder, 1936) adapted for tropical conditions (Castro Filho et al., 1998) in triplicate. Each triplicate group contained a sequence of sieves with openings of 2, 1, 0.5, 0.25,and 0.053 mm. An 80-g subsample was wet by capillarity for 10 minutes by placing it on a wetfilter paper on top of the 2 mm sieve. The volume of water placed in the reservoir was adjustedaccording to the humidity of the filter paper. The paper was then removed, and sieving was conducted by vertically shaking the set of sieves at 30 oscillations per minute for 15 minutes. The soil retained on each sieve was transferred to aluminum cans and dried at 50 °C. The soil was separated into four classes of aggregates: sand and iron concretions (> 2000 µm), macroaggregates (< 2000 µm and > 250 µm), microaggregates (<250 and >53 µm), and silt andclay (< 53 µm) as in Garland et al. (2017).

1.2.4 Nitrogen and Carbon Analysis

Total N and C were quantified in each of the aggregate classes, using an automatic elemental analyzer (Swift, 1996).

Chemical fractionation of humic substances into H, HA, and FA was carried out according to the International Humic Substances Society (Swift, 1996). Soil samples were treated with 0.1 mol L^{-1} NaOH under an N_2 atmosphere, shaken, and left to rest for 24 h. Then, they were centrifuged at 5000 g for 30 minutes. This procedure was repeated two more times. The pH of the alkaline extract was adjusted to 1.0 by dropwise addition of 20% H_2SO_4 (± 0.1) and allowed to settle for 18 h before filtration through a 0.45 µm filter. The filtrate was dilutedto 50 ml with distilled water (FA fraction). The filter was then washed with 0.1 mol L^{-1} NaOH,and the volume was adjusted to 50 ml using distilled water (HA fraction). The remaining material in the centrifuge tubes was considered the humin fraction. Quantitative determination of C fromthe FA, HA, and humin fractions was carried out by oxidation with potassium dichromate and titration with ammoniacal ferrous sulfate (Yeomans & Bremner, 1988). Contents of each fraction were transformed into kg ha^{-1} considering the soil density in each layer.

1.2.5 Data analysis

After homogeneity and normality analysis, data were subjected to ANOVA. The experimental design was a factorial in randomized blocks with four replications. Acidity amendment and N fertilization were considered fixed factors. The means of treatments were compared using the t-test ($P < 0.05$). Soil layers were not included in the statistical model, but rather analyzed independently. Total soil C and N stocks to a depth of 0-60 cm were calculatedconsidering soil bulk density and the proportion of each class of aggregate. Statistical softwareSisvar was used® and graphs were plotted using Sigmaplot®.

1.3 Results

Aggregates >2, 1-2, and 0.5-1 mm each contained ~20 Mg ha^{-1} of C, while aggregates <0.25 mm contained ~12 Mg ha^{-1} (Figure 1). Therefore, soil C was greater in aggregates >0.5 mm, when compared to smaller aggregates. In the control, soil C was lowest in the smallest aggregates (Figure 1d). However, in the presence of N, lime + gypsum application resulted in C stock 14%, 11%, and 9% greater in aggregates >1 mm, 0.5-1 mm, and 0.25-0.5 mm, respectively, compared with the control (Figure 1 b,c,d). Moreover, lime + gypsum associated with N fertilization resulted in greater C accumulation in the 0.25-0.5 mm aggregates comparedwith the other treatments in the 20-40 cm soil layer (Figure 1d). Interestingly, without N fertilization, C was lower in control and sole lime in the 0.5-1 mm, 1-2 mm, and >2 mm aggregate classes compared with lime + gypsum (Figure 1 a,b,c).

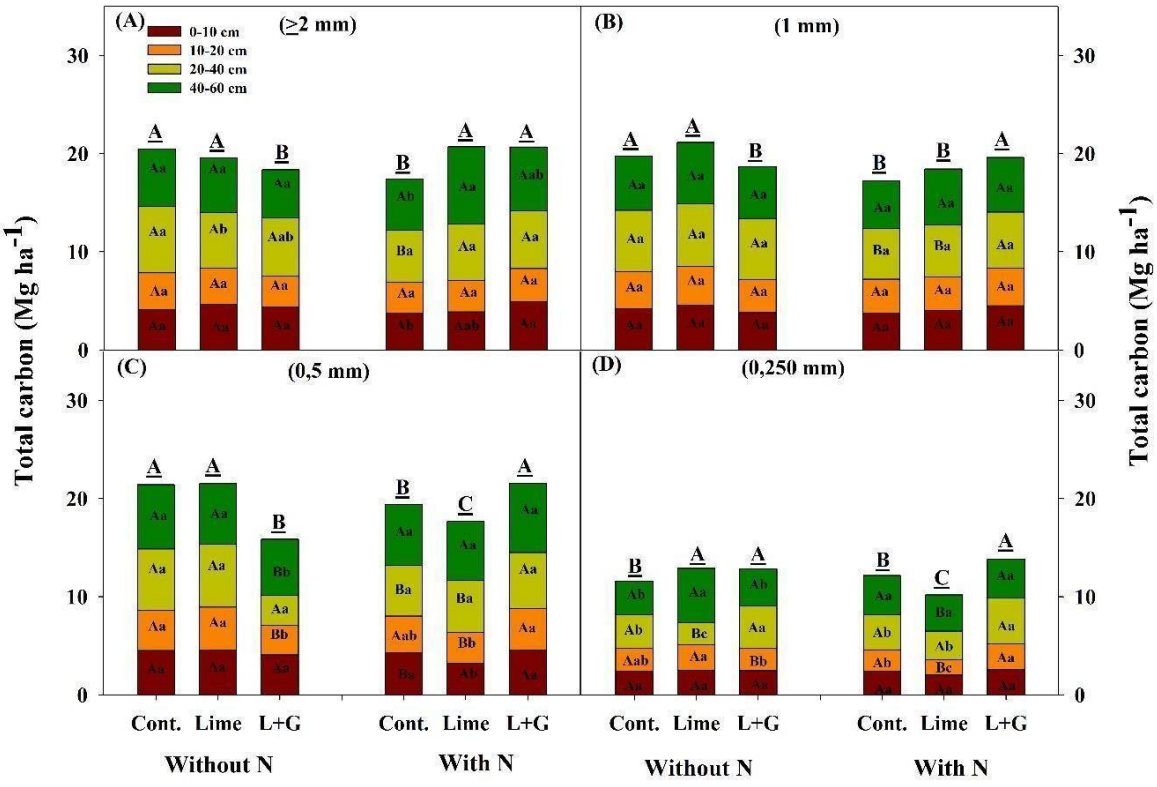

Figure 1 - Soil C stocks in soil aggregate classes and depths as affected by no correction (Cont.),lime only, lime + gypsum (L+G) and N fertilization. Different underscored capital letters abovebars show significant differences ($P< 0.05$) between treatments within a N level and soil aggregate class summed across depths. Different capital letters within bars show significant differences between treatments within a N fertilizer treatment, aggregate class, and soil depth increment. Different lowercase letters within bars show significant differences between N rateswithin corrective treatment, aggregate size class, and soil depth.

Nitrogen fertilization along with lime + gypsum applications resulted in 20% greater soil N accumulation across all aggregate classes (Figure 2). Greater soil N accumulation in aggregates of 1-2 mm and 0.5-1 mm with N fertilization (Figure 2 b, c) also occurred in the 40-60 cm depth. In the absence of N, lime + gypsum application resulted in lower N accumulationin aggregates >0.5 mm (Figure 2 a, b, c).

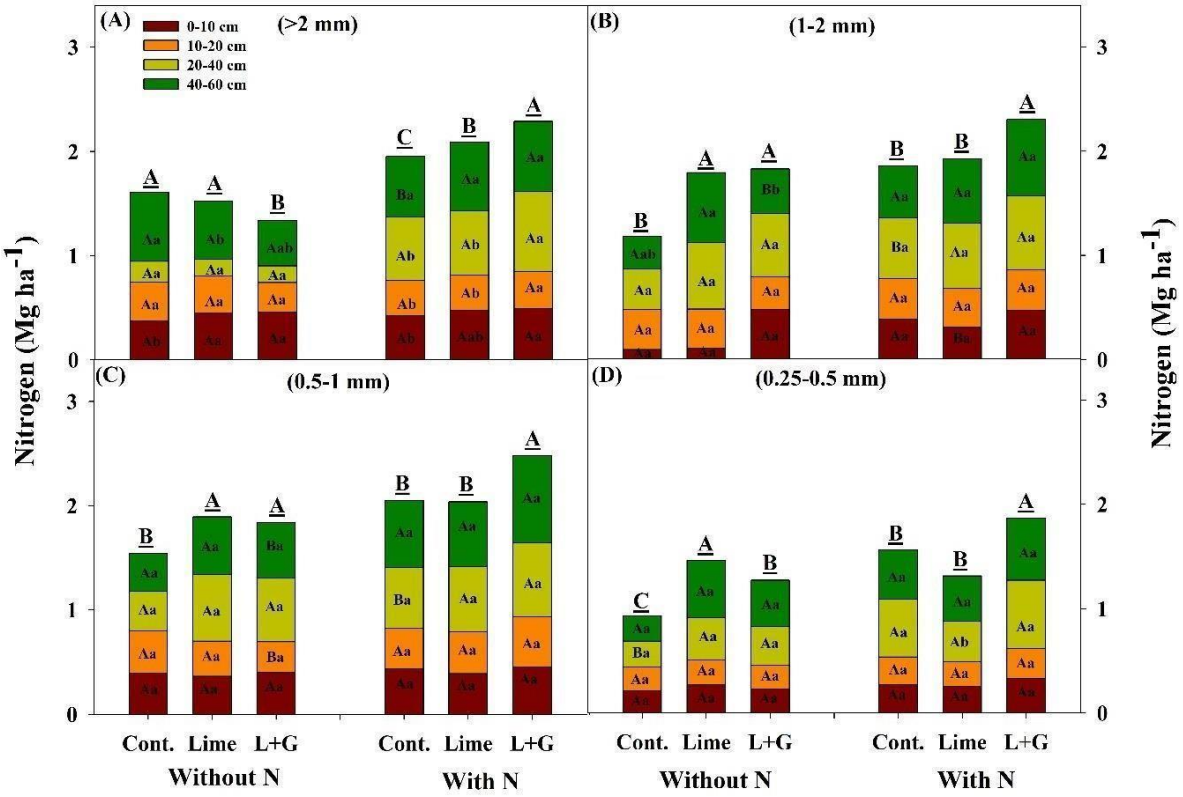

Figure 2 - Soil N stocks in soil aggregate classes and depths as affected by no correction (Cont.), lime only, lime + gypsum (L+G) and N fertilization. Different underscored capital letters above bars show significant differences ($P< 0.05$) between treatments within a N level and soil aggregate class summed across depths. Different capital letters within bars show significant differences between treatments within a N fertilizer treatment, aggregate class, and soil depth increment. Different lowercase letters within bars show significant differences between N rates within corrective treatment, aggregate size class, and soil depth.

Nitrogen fertilization associated with lime + gypsum application increased FA in the 0-10 cm layer by 55%, 59%, and 66% in aggregates >2 mm, 0.5-1 mm, and 0.25-0.5 mm, respectively. However, the effect of N fertilization in the subsurface (40-60 cm depth) was observed only in the control, with an average increase of 55% in the FA content (Figure 3).

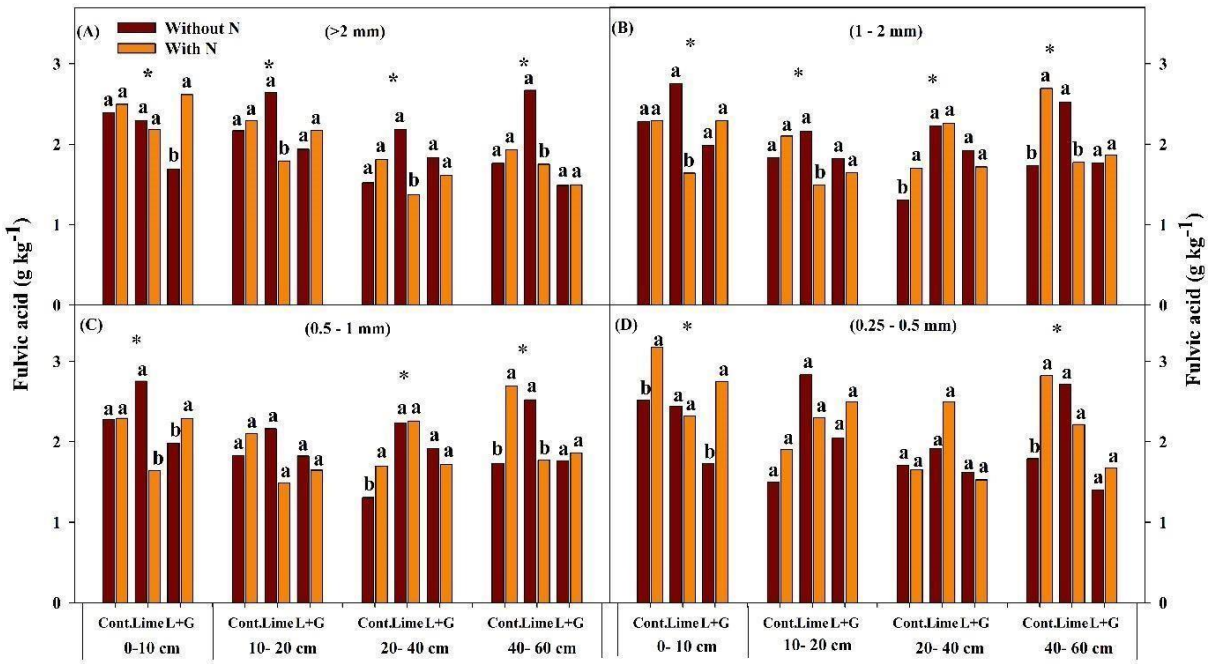

Figure 3 - Fulvic acid in soil aggregate classes and depths as affected by no correction (Cont.),lime only, lime + gypsum (L+G) and N fertilization. Lowercase letters compare the effect of N rates in each soil correction management. Asterisks show differences of soil correction management on each soil layer. *=significant at 5%.

Humic acid content was low with liming in the presence of N regardless of the aggregate class and soil depth. However, this was not observed when lime was applied with gypsum. Furthermore, in the absence of soil correction, there was no effect of N fertilization onHA (Figure 4), i.e., HA content in the soil was greater only when N associated with liming + gypsum. Additionally, the main effect of soil acidity alleviation on HA was observed on microaggregates in all soil layers (Figure 4d). When combined with liming + gypsum, HA wasgreater in the 0-10 cm soil layer compared with the absence of soil correction by 44% and 50%in the 1-2 mm and 0.5-1 mm aggregates, respectively (Figure 4 b, c).

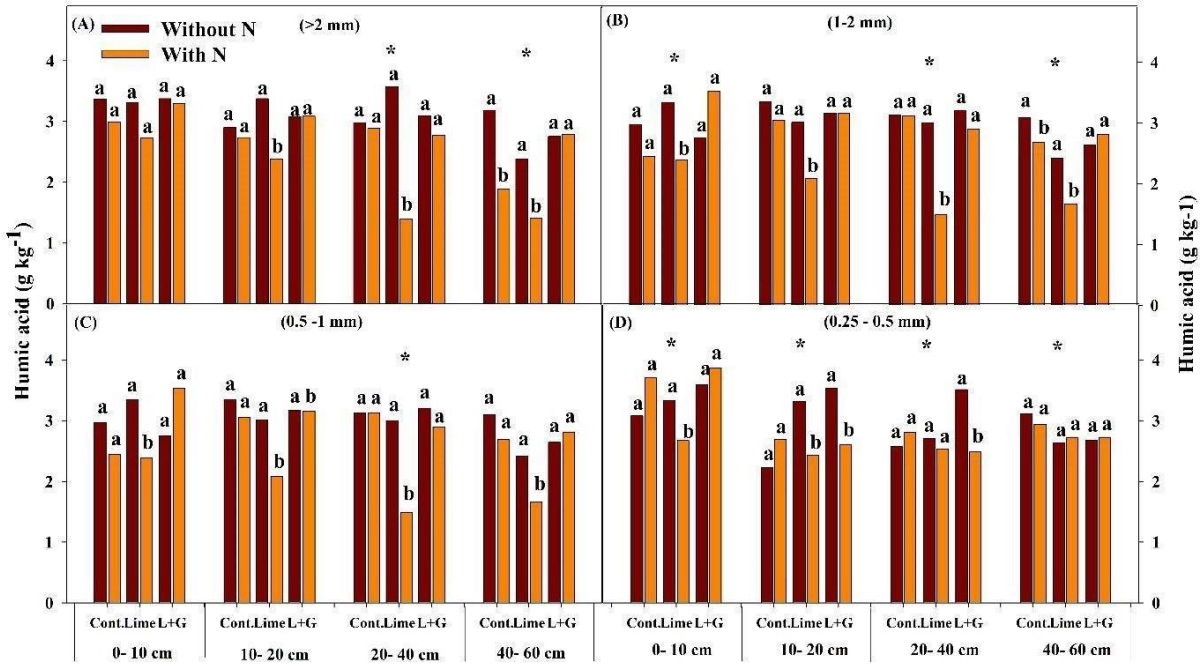

Figure 4 - Humic acid in soil aggregate classes and depths as affected by no correction (Cont.),lime only, lime + gypsum (L+G) and N fertilization. Lowercase letters compare N rates in eachsoil correction management. Asterisks show the effects of soil correction on each soil layer.
*=significant at 5%.

With N application, the alleviation of soil acidity increased humin in the surface layer only in the >2 mm and 0.5-1 mm aggregate classes (Figure 5 a, c). Liming also increased huminat the 40-60-cm depth, but only in macroaggregates (Figure 5 a). In short, the best combination to increase humin in the soil was liming associated with N fertilization.

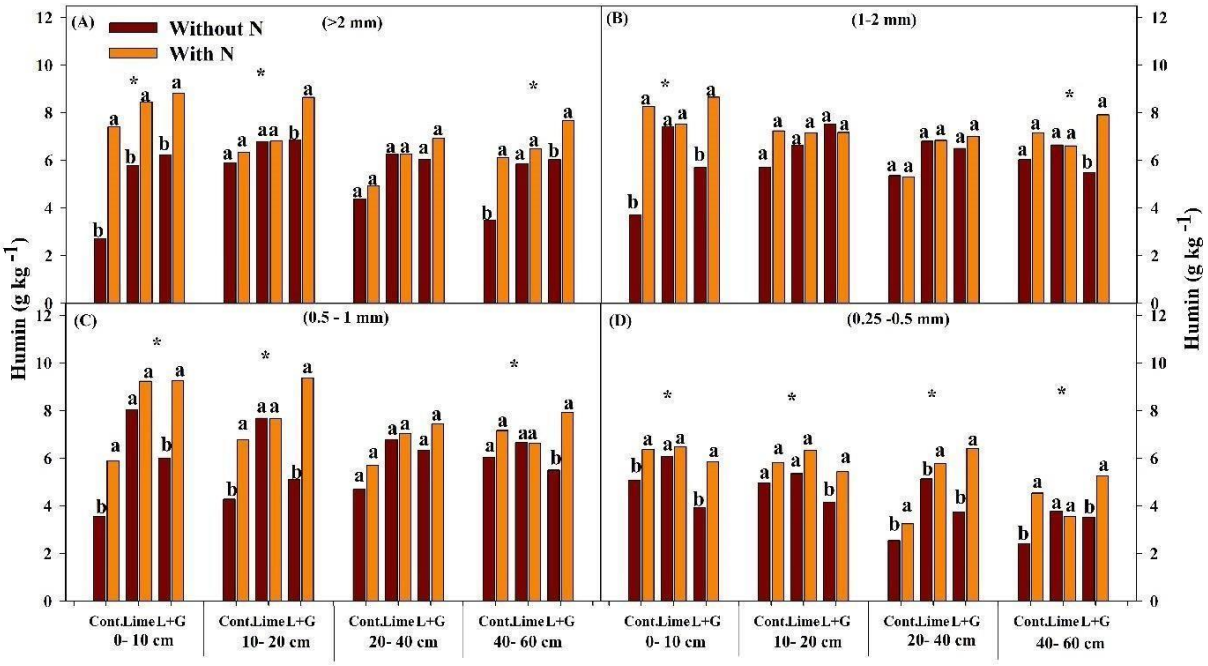

Figure 5 - Humin in soil aggregate classes and depths as affected by no correction (Cont.), limeonly, lime + gypsum (L+G), and N fertilization. Lowercase letters compare the effect of N ratesin each soil correction management. Asterisks show the effects of soil correction on each soil layer. *=significant at 5%.

The behavior of humic substances was consistent with that of humin since the latter represented 55% of the total, while humic and fulvic acids represented 26% and 19%, respectively. Liming associated with gypsum and N application increased humic substances inmacroaggregates mainly in the superficial soil layer (0-10 cm). Nitrogen fertilization increasedhumic substances by 52%, 17%, and 30% in the control, liming, and lime + gypsum, respectively, at the 0-10 cm depth (Figure 6 a). However, the effect of N fertilization at depth depended on soil correction and aggregate class, i.e., N fertilization increased humic substancesin the 40-60 cm layer only when associated with lime + gypsum (Figure 6 b, c).

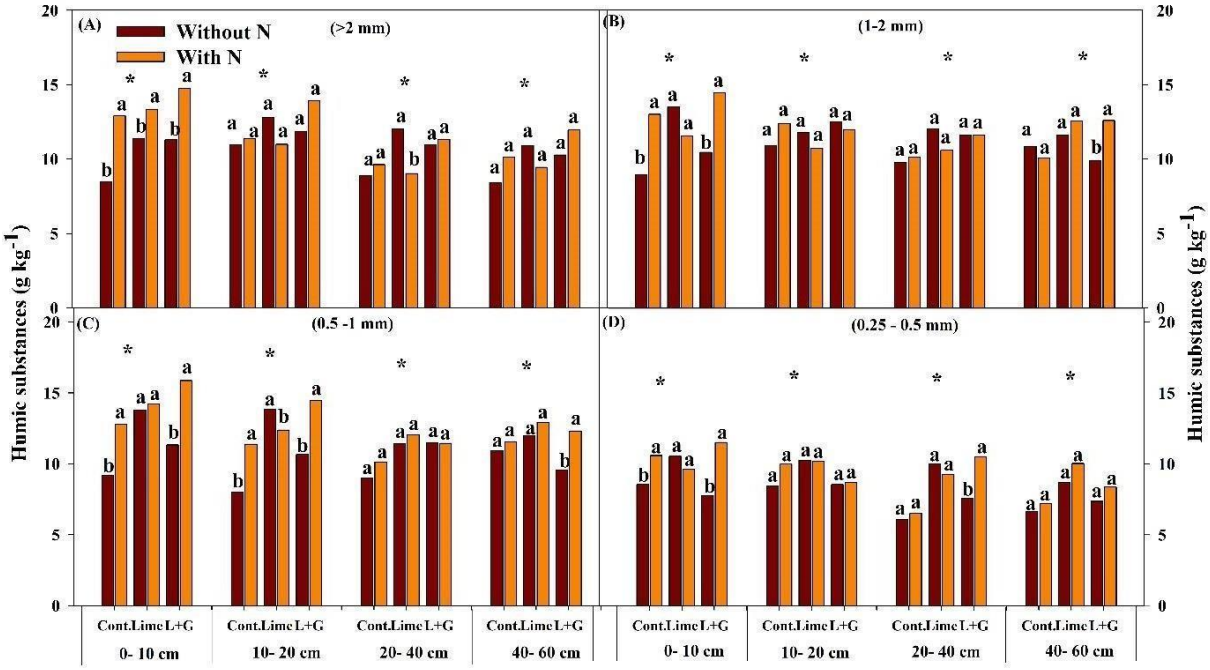

Figure 6 - Humic substances in soil aggregate classes and depths as affected by no correction (Cont.), lime only, lime + gypsum (L+G) and N fertilization. Lowercase letters compare the effect of N rats in each soil correction management. Asterisks show the effects of soil correctionon each soil layer. *=significant at 5%.

1.4 Discussion

Results support the hypothesis that combined application of lime, gypsum, and N fertilization is paramount for enhancing soil C in tropical soils and that most of this C accumulation was in macroaggregates. Since macroaggregates are sensitive to physical disruptions by tillage, the C stored may be susceptible to loss with changes in management (O'Brien & Jastrow, 2013). Thus, considering that stabilization of soil C is important from environmental and soil health perspectives, conservation agricultural practices that favor the formation of macroaggregates are recommended (Wang et al., 2019). No-till is one of these practices (Wright & Hons 2005), in addition to liming and gypsum application (Costa et al., 2021).

Gypsum can also enhance the growth of fine roots, which, in addition to forming biopores, are a source of recalcitrant compounds, important for the incorporation and storage of C in the soil (Carvalho et al., 2023). This may explain the greater C stock with additional gypsum application, especially in smaller aggregates. Despite the use of ammonium sulphate as N source, we believe this has not affected the response to lime,

gypsum and N in this experiment, because gypsum used alone has been shown to have few benefits for C sequestration (Walia et al., 2023), and sulfur concentrations in soybeans and maize were adequate (results not shown).

However, not only aggregates should be considered, but also pore connectivity, sincerecent research has shown that up to 10% of labile soil C is protected under low pore (pores > 13 µm) connectivity (Kravchenko et al., 2015). Systems under no-till are known to increase C conservation with greater pore connectivity. Therefore, this apparent paradox and the interaction of cation addition with N and increased pore connectivity in integrated cropping systems should be explored in the future, since the root systems of the tropical forage grasses, besides adding C, can improve soil pore structure (Galdos et al., 2020).

In general, soil C stock change in the cropping system with lime + gypsum and Napplication was mainly associated with greater biomass production, whether from the cash orcover crops, resulting in greater C deposition in the soil profile (Araújo et al., 2019; Costa et al., 2020). There must be a balanced addition of C and N for higher C sequestration (Cordeiroet al., 2022). Our results showed that soil C was lower mainly in aggregates >0.5 mm withliming + gypsum and no N fertilization. This may be associated with a negative N balance inthe system (Rocha et al., 2020), aggravated by greater crop yield and nutrient removal resultingfrom soil acidity alleviation. There is some controversy regarding residual N in the soil aftersoybean cropping, since the net balance may be dependent on the extent of biological N fixation(Barcelos et al. 2022). Similarly, the presence of forage grown in intercropping with maizeprevents N loss (Rosolem et al., 2017). However, in the present experiment, biological Nfixation by soybeans may not have been enough to avoid soil depletion without N fertilization.When N fertilization was combined with lime + gypsum, total soil N increased regardless of aggregate class (Figure 2). One possible explanation for this could be the greaterefficiency of biological N fixation due to better soil quality as previously reported (Bossolani et al., 2020), resulting in greater N use efficiency in a system where maize is double-croppedwith soybean. Although the application of N fertilizer to soybeans decreases its activity, biological N fixation can be low when the soil profile is very poor in N (Ciampitti et al., 2019).This was observed in a system similar to that of the present study (Souza et al., 2022). Thus, our results show that improving soil fertility with lime + gypsum was efficient at increasing soil N and keeping an appropriate C/N ratio, which allowed for C accumulation in the soil, especially when associated with N fertilization.

There are many reports on the C/N ratio relationship with C sequestration and storagein soil (Stevenson, 1994; Raphael et al., 2016; Cordeiro et al., 2022). However, this is rarely related to soil aggregation and C protection. It was recently reported that N fertilization improves microbial biomass, activity, and glomalin content in the soil, which is an aggregating agent (Cordeiro et al., 2021b). Microorganisms play a central role in the formation of micro andmacro aggregates, binding soil particles through their secretions and biomass, and soil aggregates protect C from degradation, increasing residence time (Mason et al., 2023). Therefore, future studies should evaluate the microbial community, associating this with soil aggregation and C sequestration.

Without N fertilization, FA levels were greater with liming, mainly in macroaggregates. This happened because C humification increases when soil pH is close to 6.5(Radmanović et al., 2015), which was observed in the present experiment (data not shown). Moreover, the addition of cations by lime and gypsum results in HA and FA stabilization through the formation of cationic bridges between C and the soil colloids (Mockeviciene et al., 2022). Liming increases carboxylic, alkyl, and α-aromatic compounds, thus improving the formation of soil humic substances (Trubetskaya et al., 2013). Soil pH has a direct correlation with humic substances by affecting the surface charge of organic matter and the reactivity of iron oxides. There is a positive correlation between pH and HA and humin, but a negative correlation with FA in soil aggregates (Wei et al., 2020). The negative correlation between pH and FA may explain the reduction in FA content in the surface 0-10 cm depth when combiningliming and N fertilization (Figure 3). This can be observed mainly in the long term (Mockeviciene et al.; 2022), as occurred in the present study. However, when liming was combined with gypsum applications, FA was unchanged. Walia and Dick (2018) reported that FA does not seem to be physically or chemically protected, and probably the effect of gypsum combined with lime was mainly related to the physical protection of C. Thus, the use of gypsumcan be an important tool to avoid FA loss.

Gypsum carries Ca, which is important for soil aggregation due to better physical protection (Rowley et al., 2018). In addition, it increases Ca levels in the deeper layers of the soil thus improving root growth and C incorporation in the soil profile (Araújo et al., 2019). Furthermore, greater levels of Ca in the soil profile resulting from gypsum application reduce oxygen flow, hence decreasing C mineralization (Lal, 2018). In the present study, this resultedin greater HA and humic substance levels in lower depths (40-60 cm), but only in macroaggregates and in the presence of N fertilization (Figure 4 and

Figure 6). This occurred because N fertilization with amydic or ammoniacal sources also favors the translocation of cations with depth, including Ca (Castro et al., 2015). We used ammonium sulfate as a source of N, so sulfate may also have contributed to the translocation of cations at depth. Soil amendment and N fertilization improve soil aggregation (Costa et al., 2021), which is important for the physical protection of humic substances due to limited oxygen diffusion, soil moisture, and enzyme activity (Wei et al., 2020). It has been previously reportedthat there is a tendency for humic substances to be more concentrated in macroaggregates thanin microaggregates due to their low molecular weight (Wei et al., 2020). However, in our study,this was true only for humin, because HA and FA contents were distributed proportionally between aggregate classes.

Siddique et al. (2024) reported that the greater the yield of crops, the greater the sequestration of C. However, our study shows that the form of C protected in aggregates, mainlyfrom more stable humic substances, could be more important in soil C sequestration. Furthermore, with an increase in the content of humic substances in the soil, there are improvements in the efficiency in the use of nutrients by crops and tolerance to abiotic stresses,such as drought (Gerke et al., 2022; Tiwari et al., 2023), making the cultivation system more sustainable and with lower economic risk.

1.5 Conclusion

Accumulation of C and N in soil macroaggregates resulting from lime + gypsum application depended on N fertilization input. Greater HA levels with lime + gypsum, mainly in macroaggregates, also depended on N fertilization. Nitrogen fertilization combined with lime

+ gypsum application increased humin levels thus resulting in greater accumulation of humic substances. Additionally, FA levels seemed to benefit mainly from the use of lime alone. Therefore, our hypothesis that the combination of lime, gypsum, and N application would be paramount for soil N and C accumulation was confirmed, and except for HA, aggregate size did not play a significant role.

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CHAPTER 2

IMPROVING SOIL FERTILITY, AGGREGATE STABILITY, AND YIELD IN A DOUBLE-CROPPED SYSTEM: SYNERGY OF NITROGEN, LIME, AND GYPSUM[2]

Paper submitted to Geoderma.

2.1 Introduction

Crop rotation under no-till benefits agricultural systems in several ways (Nunes, 2018),mainly by improving soil carbon (C) stocks (Cordeiro et al., 2022) and nitrogen (N) use efficiency (Rosolem et al., 2017; Gazola et al., 2023). However, these benefits are only fully realized when the soil is covered throughout the year and the cropped species have large, vigorous root systems (Souza et al., 2023). Soil acidity limits deep root growth (Vicensi et al., 2020; Souza et al., 2023) and impairs soil aggregation capacity. Applying gypsum ($CaSO_4.2H_2O$) in association with lime improves soil fertility in subsurface layers by reducing the effects of acidity, such as aluminum toxicity (Zoca and Penn, 2017; Vicensi et al., 2020), favoring deep root growth, and increasing water and nutrient uptake (Pivetta et al., 2019). A more extensive root system enables greater soil exploration and plays a crucial role in water acquisition (Lynch, 2013). Furthermore, a deeper root system allows greater N uptake by crops,greater N cycling within the system, and lower loss of N by leaching (Rocha et al., 2020; Gazola et al., 2023). However, correcting soil acidity in subsurface layers has been a major challenge in no-till systems because amendments are not incorporated (Zhao et al., 2021).

There are many advantages of double-cropping maize–soybean, such as increased yield per area and income for farmers, maximization of land and resource use, and alleviation of land burdens (Adami et al., 2018; Xu et al., 2021). However, water restriction during late fall and winter can reduce maize grain yield (Mercau and Otegui, 2014; Nóia Júnior and Sentelhas,2020).

[2] Chapter written following the guidelines of the journal Geoderm

The root system also directly affects the formation of soil aggregates, which are the basic unit of soil structure. The distribution and structure of soil aggregates determine soil physical properties, nutrient use efficiency, and soil erosion (Wang et al., 2023). Macroaggregates (> 2.00 mm) are formed by the tangle of roots and fungal hyphae (Oades andWaters, 1991) and give rise to microaggregates (0.25–0.05 mm) as they decompose Oades, 1984). Microaggregates generally comprise both particulate organic matter(POM) and mineral-associated organic matter (MAOM) resulting from the decomposition of exuded microbial compounds or microbes themselves. The outer layer of clay minerals protects the organic compounds within microaggregates from accelerated decomposition (Totsche et al., 2018).

When applied at appropriate rates, calcium (Ca) and magnesium (Mg) act as flocculating agents, favoring C stabilization (Rowley et al., 2018). Thus, the addition of Ca to the soil through lime or gypsum increases soil aggregation by up to 10 % (Chan and Heenan, 1998) by facilitating the formation of polyvalent clay-cation–organic matter complexes (Clough and Skjemstad, 2000). In addition, liming increases cation exchange capacity (CEC) in soils with variable charges, which increases cation bridging capacity (Costa et al., 2021). However, the main effects of lime and gypsum amendment on C sequestration are associated with higher production of biomass, mainly roots (Costa et al., 2021).

Although N fertilization accelerates the decomposition of organic matter, it helps stabilize C in the soil in heavier fractions (Chantigny et al., 1999; Cotrufo et al., 2019). N fertilization increases the amount of C in the rhizosphere, probably due to greater rhizodeposition and greater root biomass (Liu et al., 2019). The greatest incorporation of C under N fertilization occurs in large aggregates (0.25–2.00 mm) (Luo et al., 2019). In intensivesystems, N fertilization can acidify the soil (Hao et al., 2020). Correcting soil pH through limingincreases biological N fixation (Alves et al., 2021) and reduces the response potential of crops to N application, especially soybean (Hammerschmitt et al., 2021). Furthermore, crop residue decomposition increases as the soil pH approaches neutral values, which may reduce the mineral N response.

Collectively, the effects of liming, gypsum application, and N fertilization synergistically influence the N cycle in the soil and can ultimately improve N acquisition and crop yield (Chaganti et al., 2019). Applying lime in association with gypsum to no-till croppingsystems may not only promote plant resilience to drought but also reduce the need for N fertilization (Shafiq et al., 2021).

Although some effects of soil acidity correction and N fertilization in integrated agricultural production systems are known, most studies have evaluated only the surface layer of the soil. Studies of the synergistic effects of applying lime and gypsum in association withN fertilization to soybean double-cropped with maize intercropped with forage on soil fertility and aggregates, especially in deeper soil layers, are rare. The objective of this study was to evaluate these effects. We hypothesized that (i) liming associated with gypsum application alleviates soil acidity in deeper layers, improving soil aggregation; (ii) improving soil aggregation and fertility in deeper layers increase N uptake and reduces the need for Nfertilization, and (iii) improving soil aggregation and N acquisition increases crop yields

2.2 Material and methods

2.2.1 Characteristics of the study area

The study was carried out under field conditions in Botucatu, São Paulo, Brazil (coordinates 22° 49'51.6" S 48° 25'38.6" W, altitude 780 m). The climate is Cwa according to Köppen's classification, i.e., mesothermal with dry winters. The average annual temperature is 20.7°C, with an average monthly maximum of 26.1°C and an average monthly minimum of 15.3°C. Annual precipitation is 1696 mm. The soil is a Typic Haplorthox (Soil Survey Staff, 2014). Selected chemical characteristics and particle sizes of the soil are presented in Table 1. The experiment began in October 2016 to evaluate the response of soybean double-cropped with maize intercropped with Guinea grass (*Megathyrsus maximum*, cv Tanzania) to liming, liming and phosphogypsum application, and N fertilization. In this study, we evaluated the 2021and 2022 seasons.

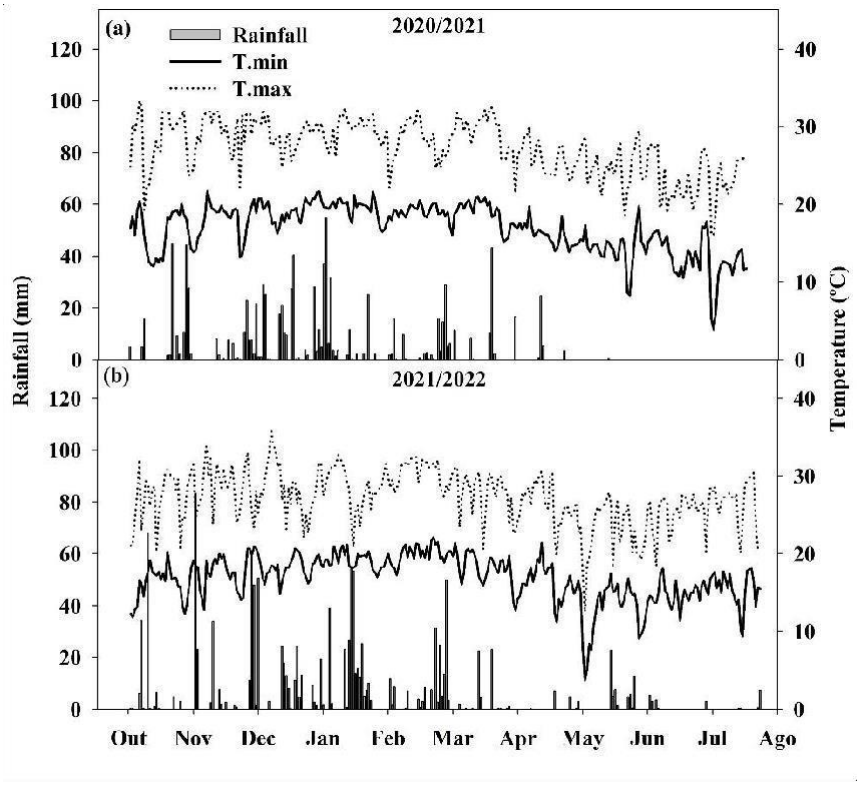

Fig. 1 - Precipitation and monthly maximum and minimum temperatures during the soybeanand maize harvests in 2020/2021 and 2021/2022.

Table 1 - Chemical characteristics and particle size of the soil in which the experiment was conducted August 2016.

Layer	pH	OC	P_{resin}	S	Al	K	Ca	Mg	CEC	Sand	Clay
	$CaCl_2$	g kg^{-1}	mg kg^{-1}			mg kg^{-1}			$mmol_c$ kg^{-1}	g kg^{-1}	
0-10 cm	4.3	10.2	12.8	4.8	84	81.9	238	141	78.2	540	343
10-20 cm	4.2	8.1	10.1	4.7	81	42.9	188	90	65.6	520	368
20-40 cm	4.1	7.1	11.4	4.8	111	19.5	74	49	63.1	518	410
40-60 cm	3.9	6.3	5.9	10.8	180	15.6	68	42	97.0	510	420

OC = organic carbon; Presin: Phosphorus extracted by ion exchange resin; CEC = cation exchange capacity.

2.2.2 Experimental planning and treatments

The experimental design was conducted in a 3 x 4 factorial scheme in complete randomized blocks with four replicates. The first factor comprised three treatments: a

control, soil acidity alleviation with lime (L), and soil acidity alleviation with lime plus, phosphogypsum(L+G). The second factor consisted of four N rates of 0, 80, 160 and 240 kg ha^{-1}. Lime and agricultural, phosphogypsum were applied at rates of 2.9 Mg ha^{-1} and 2.0 Mg ha^{-1}, respectively,before soybean planting in 2016, 2018, and 2020. The N rates were applied annually to maize as ammonium sulfate (20% N, 22% S). The lime rate was originally calculated to raise soil basesaturation (BS) to 60% in the 0-20 cm layer, and the agricultural, phosphogypsum rate was calculated by multiplying the clay content in the 20–40 cm layer by 6.0 (Quaggio and Van Raij,1997).

The N fertilizer was split as 30 kg ha^{-1} at planting and the remainder broadcast at maizestage V7/V8. Soybeans were always planted in spring/summer (October-November) and maize
+ guinea grass as an intercropping system was planted in the fall (March). All plots were 4.5 mwide and 10.0 m long.

2.2.3 Crop management

Soybean (cv. TMG 7062 IPRO; Tropical Improvement and Genetics) was cropped in spring–summer (October–February) in both years. Two weeks before soybean planting, spontaneous vegetation, mainly grass weeds, was terminated with glyphosate (2.30 kg^{-1} a.i.). Soybean was planted over the residues on the soil surface in rows 45 cm apart to reach a population of 330,000 plants ha^{-1}. Maize (Dow AgroSciences Hybrid 2B 587 RRBTPW) was cropped in fall–winter (March–June) after the soybean harvest each year. Maize was sown to reach a population of 55,000 plants ha^{-1} and intercropped with Guinea grass.

Soybean was fertilized annually at sowing with 35 kg ha^{-1} P as triple superphosphate and 83 kg ha^{-1} K as KCl, and the seeds were inoculated with *Bradyrhizobium* spp. solution on the day of sowing (60 ml of a.i. per 50 kg of seeds). Maize received 35 kg ha^{-1} P as triple superphosphate, 82 kg ha^{-1} K as potassium chloride, and 30 kg ha^{-1} N as ammonium sulfate at sowing. At the V7/V8 stage, maize was side dressed with 41 kg ha^{-1} K and 0, 80 120, or 240 kgha^{-1} N (depending on the treatment) at 0.1 m from the row.

Maize was harvested at physiological maturity (R6) in July 2019 and 2020, and soybean was harvested at the R8 stage in March 2020 and February 2021. For both crops, 10 mof the 4 central rows of each plot were harvested to estimate grain yield, which was

calculated based on the relationship between the grain mass of each plot and its respective water content (adjusted to 130 g kg^{-1} water).

2.2.4 Leaf analysis

The leaf macro- and micronutrient contents of soybean and maize were determined from samples of 10 leaves collected from each field plot. For soybeans, the first fully expanded leaf on the main axis was sampled at the R2 development stage (Chapman et al., 1962). For maize, the leaf opposite the reproductive structure was sampled at the R1 stage (Chapman et al., 1962). The sampled plant material was placed in paper bags and dried to constant weight ina forced-air oven at 65ºC. The leaf samples were then ground in a Wiley mill. N content was determined by Kjeldahl distillation after acid digestion. The contents of the other nutrients were determined by atomic absorption spectrophotometry after double acid digestion.

2.2.5 Soil sampling

In October 2020 and 2021, before soybean planting, three soil subsamples at depths of0-10, 10-20, 20-40, and 40-60 cm were randomly taken in each plot from the wall of a trench with a width of 40 cm, and length of 50 cm, where the soil was friable. Subsamples at the samedepth were combined to form a composite sample for each plot. Monoliths with a 15 x 20 cm horizontally and 10 cm vertically were collected using trowels. The samples were placed in plastic bags and transported to the laboratory. To analyze soil fertility attributes, five subsamples were taken from each plot at the end of the study, after the 2022 maize harvest.

2.2.6 Soil aggregates

The monoliths were gently fragmented and classified using a set of overlapping sieves ranging from 8-mm to 4-mm mesh. The soil retained in the 4-mm sieve was dried at 50°C for 48 h in a forced-air oven to homogenize the moisture. The dried soil was then separatedinto aggregate classes in triplicate by wet screening (Yoder, 1936) adapted for tropical conditions (Castro Filho et al., 1998) using a set of overlapping sieves with mesh sizes of 2, 1, 0.5, and 0.25 mm. Before sieving, an 80-g subsample was moistened by

capillarity by placingit on wet filter paper over a 2-mm sieve for 10 min. The volume of water in the equipment's reservoir was adjusted so that the water level was sufficient to keep the filter paper moist but did not directly contact the soil samples. The paper was then removed, and sieving was conducted by vertical oscillation for 15 min at 30 cycles per minute. The soil retained on each sieve was transferred to aluminum cans and dried at 50°C, and the mass was measured on a precision scale (0.001 g). The soil was separated into three classes of aggregates as in Garland et al. (2017): >2.00 mm (macroaggregates), 1.00–0.50 mm (mesoaggregates), and >0.25 mm (microaggregates).

Based on each aggregate mass, the weighted average diameter (WAD) and geometricmean diameter (GMD) were determined using equations 1 and 2:

$$WAD = \Sigma n\ (xi \cdot wi) \quad (1)$$

where xi is the average diameter of the classes and wi is the proportion of each class in relation to the total; and

$$GMD = \exp \sum n\ wp \cdot \log xi \,/\, \sum n\ wi \quad (2)$$

where wp is the weight of the aggregates of each class (g).

Equation 3 was used to calculate the aggregate stability index (ASI):

$$ASI = [(dry\ sample\ weight - wp25) \,/\, dry\ sample\ weight] 100 \quad (3)$$

where wp25 is the weight of aggregates smaller than 0.25 mm. The ASI represents thegeneral aggregation of the soil without considering the distribution of aggregates into classes.

2.2.7 Soil Fertility

The soil samples were analyzed according to the methodology described by Raij et al.(2001). Briefly, pH was determined in a 1:2.5 suspension (soil:solution) in 0.01 M $CaCl_2$; soil organic matter (SOM) was determined by dry combustion; available phosphorus (P), exchangeable potassium (K), Ca, and Mg contents were extracted by resin; and available sulfur
(S) content was determined using $Ca(K_2PO_4)_2$ solution. Available contents of copper (Cu), iron(Fe), manganese (Mn), and zinc (Zn) were determined by extraction in extraction by diethylenetriamine pentaacetate (DTPA) at pH 7.3, and boron (B) content was determined by hot water extraction.

2.2.8 Statistics

After testing the homogeneity of errors and data normality using the Levene test and Shapiro–Wilk test, respectively, analysis of variance (ANOVA) was performed considering treatments as fixed effects and blocks as random effects. When the F-test value from ANOVA was significant, means were compared using the t test at a 5% probability level of error. Sisvar® statistical software was used. Graphs were plotted using SigmaPlot®.

2.3 Results

2.3.1 Grain yield

In general, soybean yields were higher in L and L+G than in the control (Fig. 2A and 2B), and when N was applied to the system, soybean yields were not significantly higher in L+G than in L. Although N fertilization of maize at 80 kg ha^{-1} increased soybean yield in the control in the first season (2020/2021), N application to maize generally decreased soybean yields, particularly in the second season (Fig. 2B), regardless of soil pH correction.

Maize yields were responsive to liming and, phosphogypsum application (Fig. 2C and2D). Interestingly, the increase in maize yields in L+G compared with the control decreased asthe N rate increased. In the absence of N, maize yields increased by 90% (2020/2021) and 45%(2021/2022) in L+G compared with the control. However, when 240 kg ha^{-1} N was applied, maize yields increased by only 16% and 18% in the first and second seasons, respectively, in L+G compared with the control.

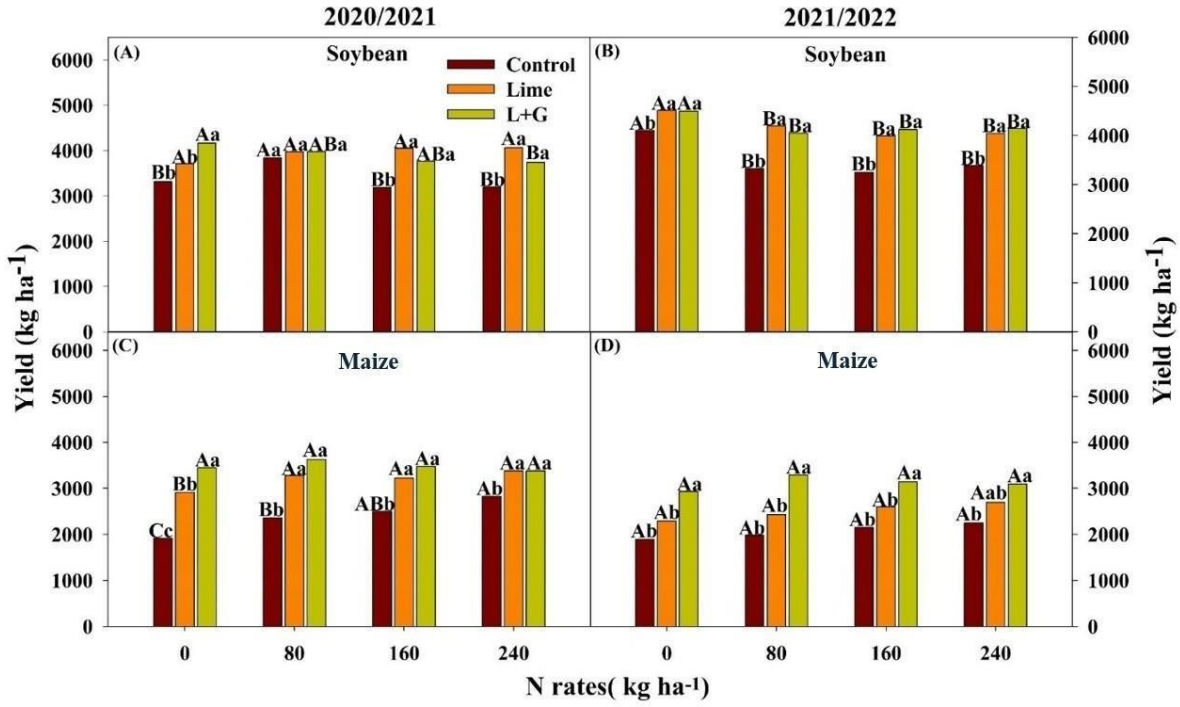

Fig. 2 - Soybean and maize grain yield, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters comparesoil correction management at each N dose.

2.3.2 Leaf nutrient content

Because there was no effect of season on nutrient contents in soybean and maize leaves, the averages of the two seasons are discussed. Soybean and maize leaf N contents werehigher in L than in the control, especially in the absence of N fertilization, and N fertilization rates greater than 160 kg ha^{-1} did not further increase leaf N content (Table 2). L+G did not increase leaf N content compared with L except in maize in the absence of N fertilization.

Interestingly, L+G increased soybean leaf P content compared with L but did not affect maize leaf P content (Table 2). At low N fertilizer rates, soybean leaf K content was lower in L than in L+G. Surprisingly, the treatments did not affect soybean and maize leaf Ca contents,but in the absence of N fertilization, L increased soybean and maize leaf Mg contents (Table 2). L+G increased maize leaf S content in the absence of N fertilization and in the presence of fertilization with up to 80 kg ha^{-1} N, whereas soybean leaf S content was increased only by L+G(Table 2). The effects of the treatments on the leaf contents of micronutrients were small, but L+G reduced the leaf contents of Fe and Mn in both crops (Table 2).

Table 2 - Average leaf contents of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, iron, manganese, zinc, and copper for the 2020/2021 and 2021/2022 harvests for soybean and maize crops. Capital letters compare N doses in each soil correction management (i.e., lines comparison for each column). Lowercase letters compare soil correction management at each N dose (i.e., columns comparison for each line).

N rates (kg ha^{-1})	Soybean			Maize		
	Control	L	L+G	Control	L	L+G
			Nitrogen (g kg^{-1})			
0	41.0 Cb	53.4 Aa	51.5 Ba	19.6 Bc	22.09 Ab	23.1 Aa
80	42.0 Bb	51.7 Aa	51.8 Aa	26.5 Aa	26.0 Aa	26.5 Aa
160	51.3 Aa	52.0 Aa	53.0 Aa	27.8 Aa	27.6 Aa	27.6Aa
240	52.5 Aa	54.8 Aa	54.8 Aa	27.9 Aa	28.5Aa	29.2 Aa
			Phosphorus (g kg^{-1})			
0	3.8 Ab	4.6 Aa	5.5 Aa	1.5 Ba	1.9 Aa	1.6 Aa
80	4.3 Ab	4.4 ABb	5.6 Aa	1.5 Bb	1.6 Aab	2.2 Aa
160	4.1 Ab	3.4 Bb	5.4 Aa	1.8 Aa	2.4 Aa	1.3 Bb
240	4.1 Ab	4.2 ABb	5.3 Aa	1.6 Ba	1.9 Aa	1.7 Aa
			Potassium (g kg^{-1})			
0	22.3 Aa	17.3 Bb	23.4 Aa	26.5 Aa	27.3 Aa	27.4 Aa
80	25.2 Aa	17.0 Bb	26.2 Aa	25.8 Aa	28.0 Aa	28.4 Aa
160	26.4 Aa	18.4 Bb	26.0 Aa	28.1 Aa	24.6 Aa	27.6 Aa
240	24.9 Aa	21.4 Aa	25.1 Aa	27.7 Aa	26.4 Aa	26.4 Aa
			Calcium (g kg^{-1})			
0	11.9 Aa	14.1 Aa	15.2 Aa	9.8 Aa	12.3 Aa	13.0 Aa
80	12.5 Aa	13.8 Aa	15.5 Aa	10.5 Aa	11.8 Aa	12.3 Aa
160	12.4 Aa	12.9 Aa	15.7 Aa	11.6 Aa	12.5 Aa	12.9 Aa
240	11.3 Ba	14.7 Aa	15.0 Aa	12.5 Aa	11.2 Aa	8.7 Bb
			Magnesium (g kg^{-1})			
0	2.1 Bb	2.7 Aa	2.4 Ba	1.4 Bb	2.6 Aa	1.7 Ba

80	2.7 Aa	2.7 Aa	2.7 Aa	1.9 Ab	2.0 Aa	1.9 Ab
160	2.9 Aa	2.5 Aa	2.5 ABa	1.4 Bb	2.2 Aa	1.9 Aab
240	2.9 Aa	2.8 Ba	2.5 ABa	1.1 Cb	2.1 Aa	1.7 Ba
			Sulfur (g kg^{-1})			
0	1.8 Ac	1.9 Ab	2.0 Aa	1.1 Bb	1.2 Bb	1.7 Aa
80	2.4 Aa	2.5 Aa	2.8 Aa	1.2 Bb	1.4 Aab	1.5 Aa
160	2.5 Aa	2.5 Aa	2.5 Aa	1.3 Ab	1.5 Aab	1.7 Aa
240	2.5 Aa	2.3 Aa	2.3 Aa	1.4 Ab	1.7 Aa	1.6 Aab
			Boron (mg kg^{-1})			
0	32.5 Aab	31.7 Aa	33.7 ABa	29.3 Aa	33.0 Aa	33.2 Aa
80	32.5 Aab	32.6 Aa	32.8 Ba	31.6 Aa	33.3 Aa	33.9 Aa
160	34.3 Aa	34.8 Aa	37.2 Aa	32.7 Aa	33.0 Aa	35.4 Aa
240	27.4 Bb	34.4 Aa	32.3 Ba	31.9 Aa	33.0 Aa	33.6 Aa
			Iron (mg kg^{-1})			
0	178.3 Aa	84.9 Cc	134.7 Ba	403.7Aa	299.7 Ba	263.6 Ba
80	194.3 Aa	147.4 Ba	146.9 Ba	316.1 Ab	319.2 Aa	327.8 Aa
160	203.9 Aa	137.1 Bb	155.4 Ba	288.7 Aa	342.9 Aa	319.9 Aa
240	169.6 Aa	183.7 Aa	167.7 Aa	350.1 Ab	319.6 Aa	315.5 Aa
			Manganese (mg kg^{-1})			
0	188.2 Aa	101.5 Bb	89.0 Cc	80.0 Bb	51.4 Cb	82.6 Bb
80	149.5 Ab	103.8 Bb	89.7 Cc	117.6 ABb	66.1 Cab	76.2 Bb
160	154.4 Ab	109.2 Bb	102.6 Bb	107.4 ABab	90.9 Aa	101.1 Aab
240	146.2 Bb	188.5 Aa	144.0 Aa	163.6 Aa	81.3 Ba	113.7 Aa
			Zinc (mg kg^{-1})			
0	32.3 Aa	25.6 Aa	22.1 Aa	26.6 Ab	24.2 Ab	27.5 Aa
80	33.1 Aa	26.8 Aa	22.2 Aa	32.3 Aa	27.7 Aa	27.4 Aa
160	38.5 Aa	32.7 Aa	28.2 Aa	26.8 Ab	30.0 Aa	31.7 Aa
240	26.0 Aa	25.0 Aa	24.1 Aa	33.8 Aa	30.4 Aa	30.7 Aa
			Copper (mg kg^{-1})			
0	8.4 Aa	4.8 Ab	7.4 Aa	7.8 Aa	8.7 Aa	8.4 Aa
80	7.7 Aab	6.5 Aa	7.6 Aa	9.6 Aa	8.6 Aa	9.0 Aa
160	8.1 Aa	6.6 Aa	7.4 Aa	7.9 Aa	9.8 Aa	9.7 Aa

240	6.6 Bb	6.5 Aa	8.1 Aa	9.1 Aab	9.4 Aa	9.8 Aa

L: lime; L+G: lime+gypsum.

2.3.3 Aggregates

L increased soil aggregate stability down to the 20–40 cm layer in the first season andthe 40¬–60 cm layer in the second season (Fig. 3). Compared with L, L+G provided no additional benefit for soil aggregate stability. N application at rates greater than 80 kg ha^{-1} reduced soil aggregate stability in deeper layers in the second season (Fig. 3F and 3H).

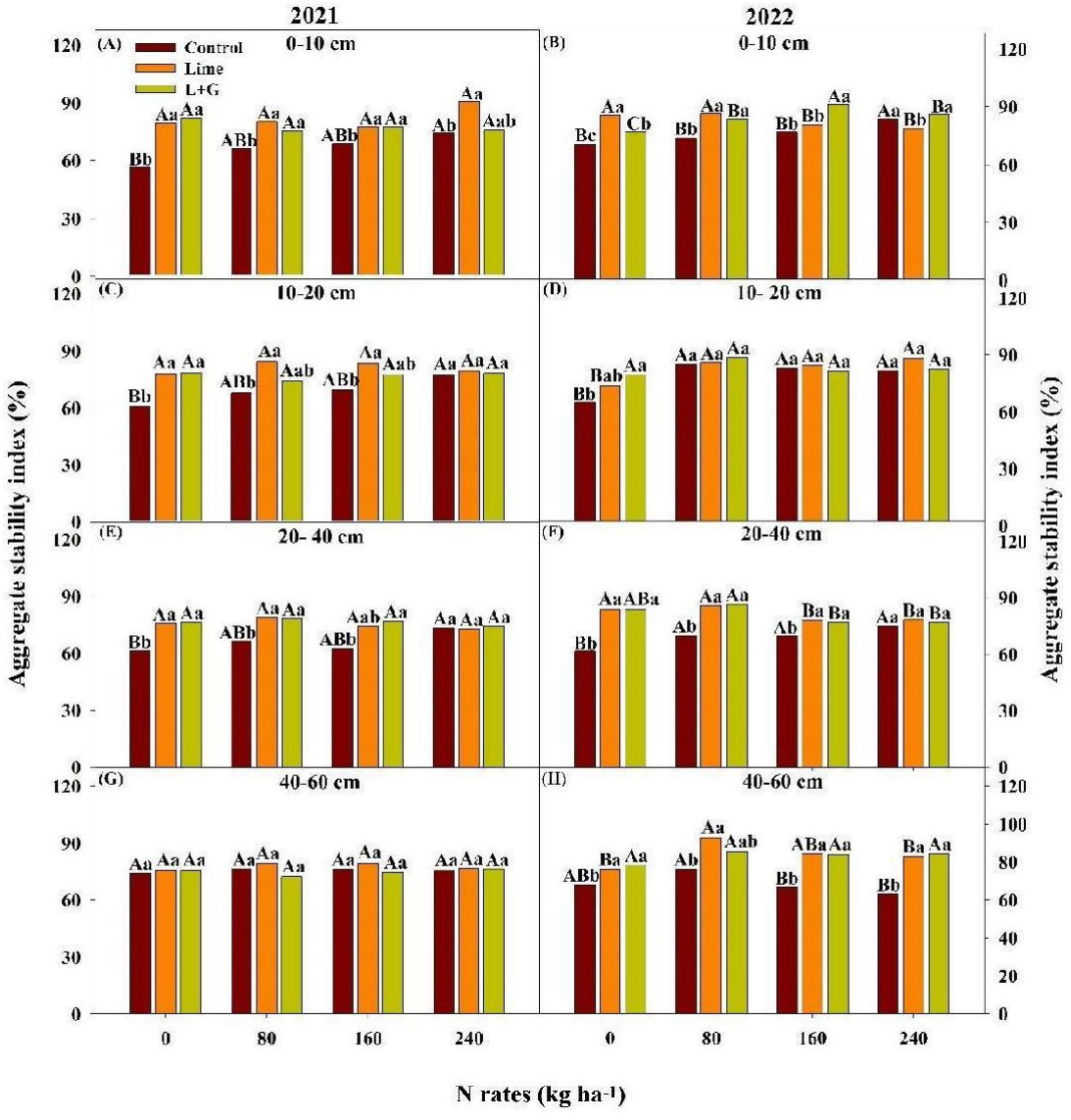

Fig. 3 - Aggregate stability index, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters comparesoil correction management at each N dose.

By contrast, a strong impact of L+G on GMD was observed, mainly in the surface layer of the soil (Fig. 4A and 4B) in association with N rates of 0–160 kg ha^{-1}. Notably, the large changes in GMD on the surface were not influenced by increases in the N rate

(Fig. 3A and 3B). In the surface layer, GMD increased in the control treatment as the N rate increased, whereas, in L+G, GMD was highest at an N rate of 160 kg ha^{-1}. Interestingly, the increase in GMD in the surface layer at the highest N rate was greater in control than in L+G. L+G alsoincreased GMD in deeper layers, but to a lesser extent than in the surface layer.

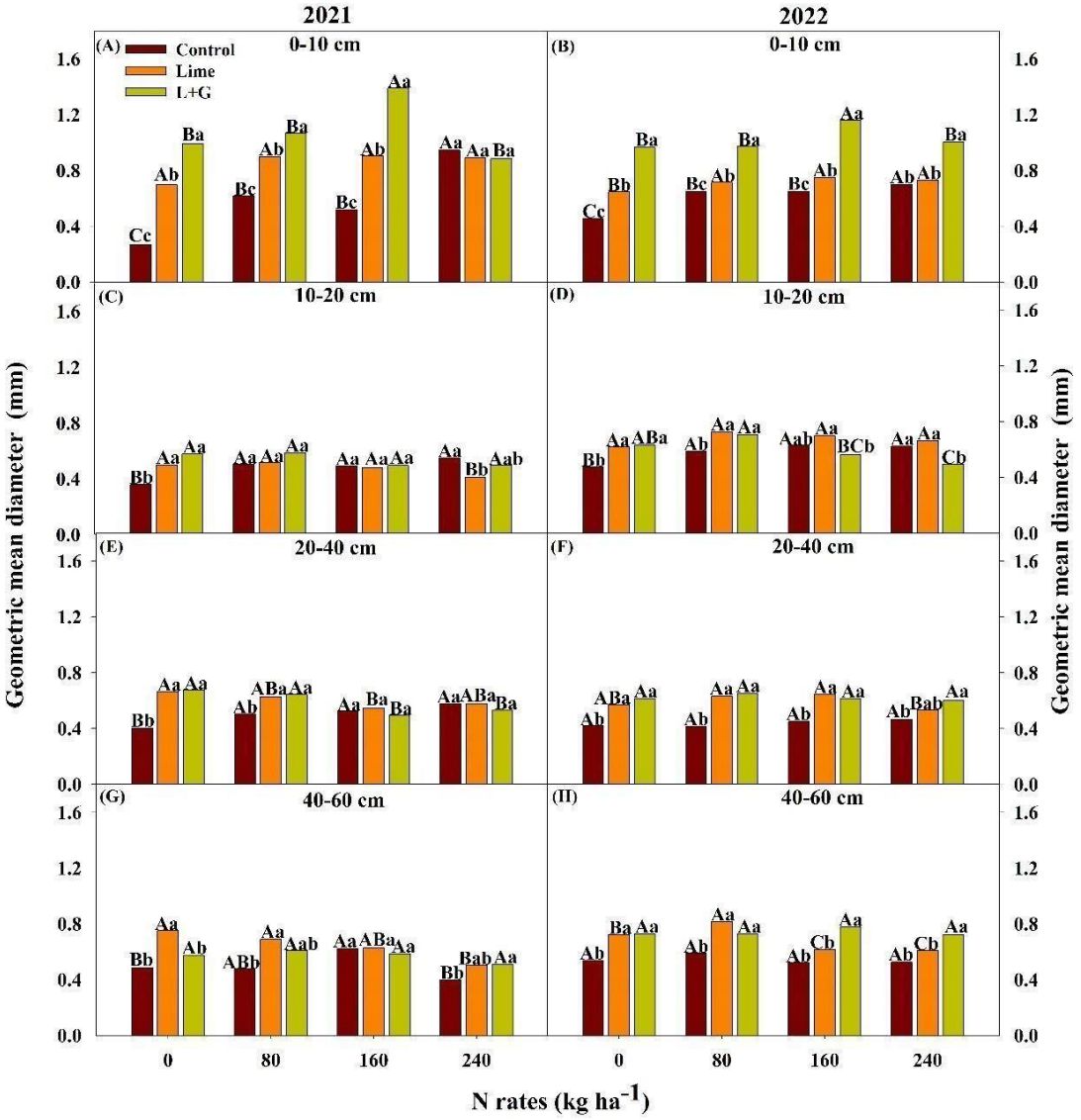

Fig. 4 - Geometric mean diameter, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters comparesoil correction management at each N dose.

The treatments affected WAD up to the 40–60 cm soil layer (Fig. 5). The impact of L+G on WAD was greater at the lowest N rates. At the highest N rate, WAD decreased in

L+G, especially in the surface layer. The greatest positive effects of L+G on WAD were observed inthe 0–10 cm layer at N rates up to 160 kg ha^{-1} (in both seasons) and in the 40–60 cm layer at Nrates above 160 kg ha^{-1} in the second season.

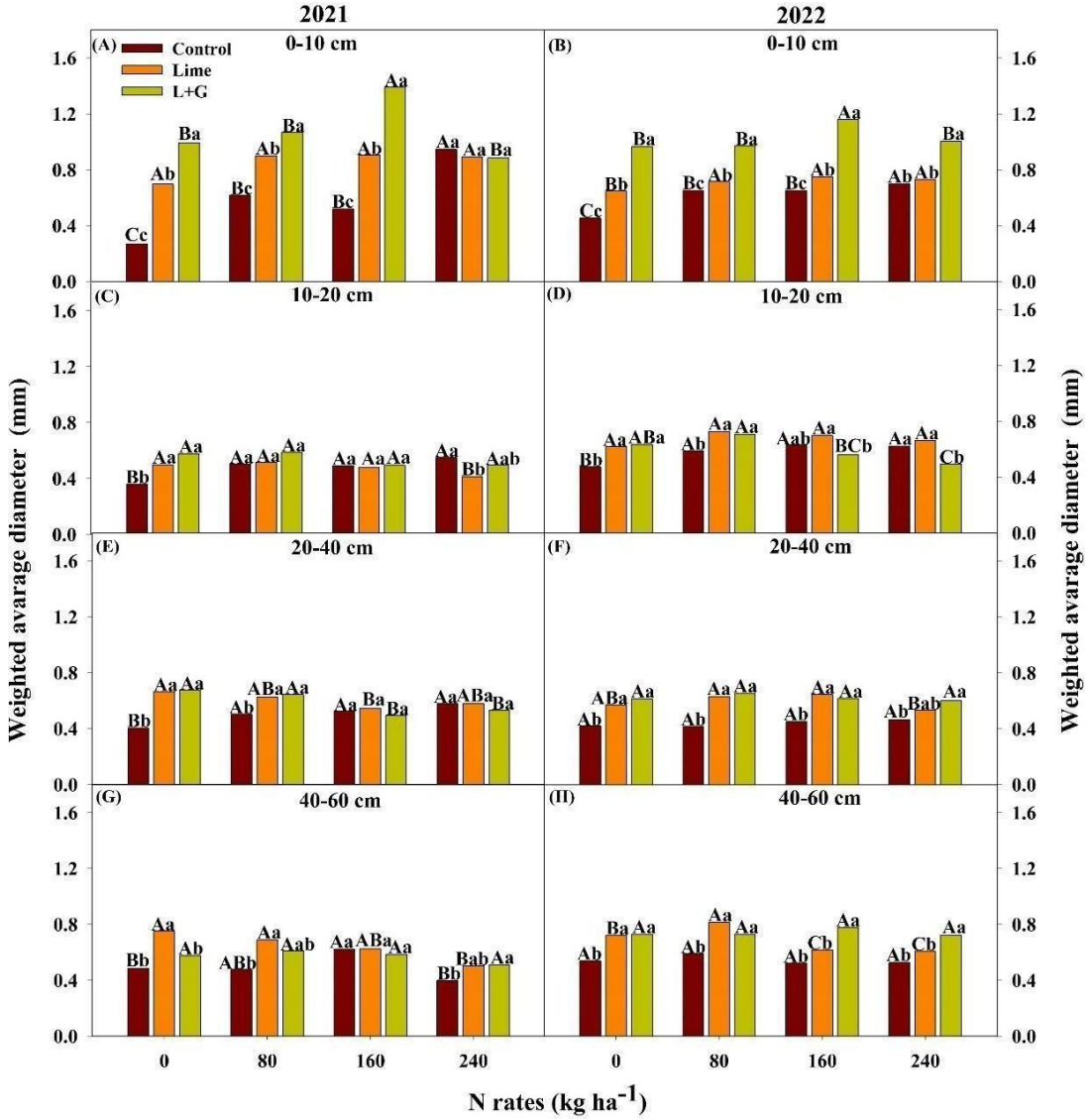

Fig. 5 - Weighted average diameter, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters comparesoil correction management at each N dose.

2.3.4 Soil fertility

After the maize harvest in 2022, the effects of the treatments on soil pH were observed only in the surface layer of the soil (Fig. 6A). Soil pH was highest in L+G in the

absence of N fertilization or in the presence of low rates of N. L+G considerably increased the availability ofCa in the soil, especially in the surface layer (Fig. 6B). Compared with the control, L+G increased Ca availability up to the 40–60 cm layer, mainly at low N rates (Fig. 6H). In general,N fertilization acidified the soil when soil pH was higher (i.e., in L or L+G, Fig. 6). Under conditions of strong soil acidity (control treatment), N fertilization did not aggravate soil acidity(Fig. 6).

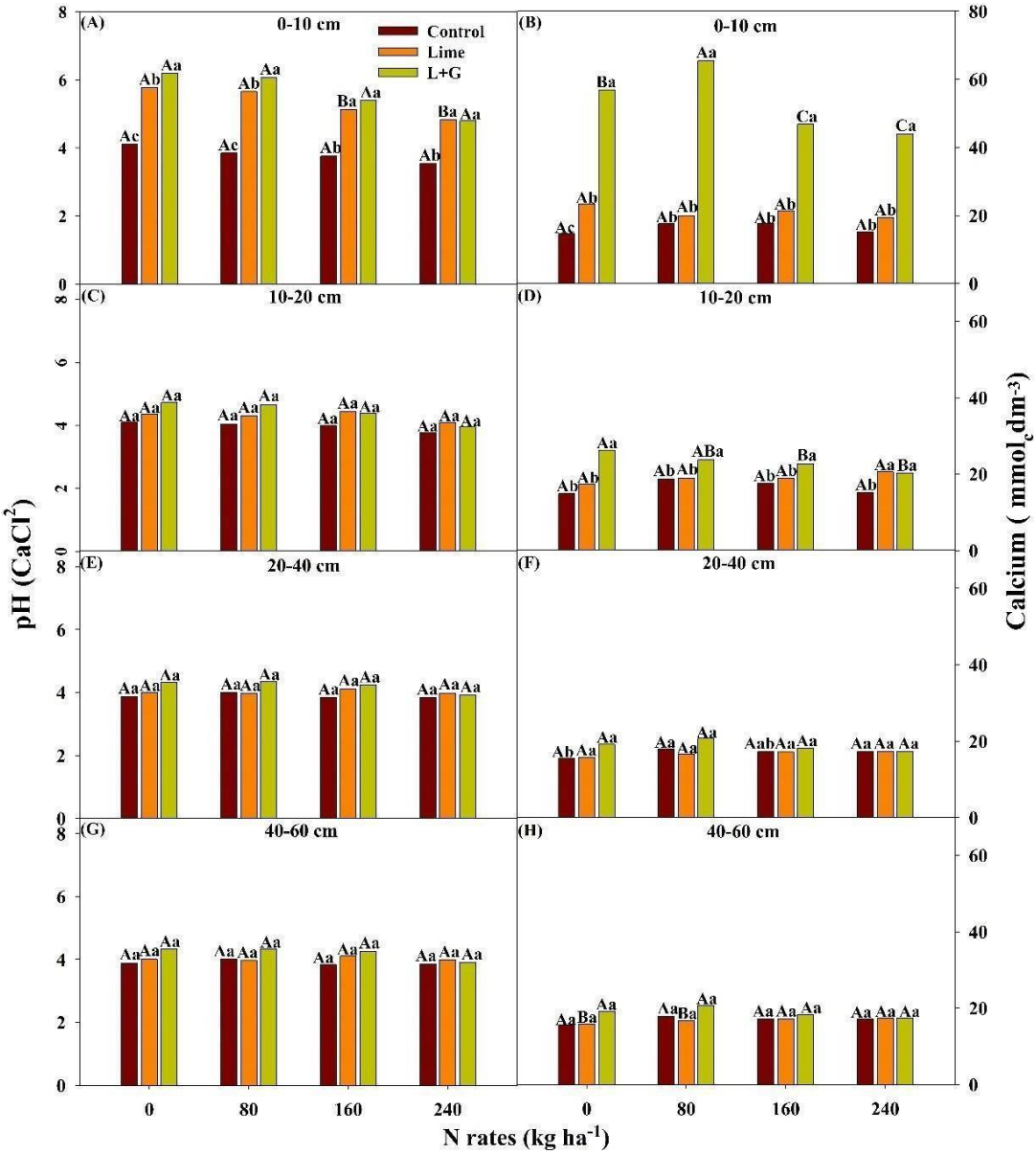

Fig. 6 - pH and calcium (Ca) content in the soil, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters compare soil correction management at each N dose.

In scenarios with low or no N, L increased soil P content, primarily in the 0–10 cm layer, whereas soil P content decreased at higher N rates (Fig. 7). At soil depths greater than 40 cm, soil P content did not differ significantly among the treatments. In the surface layers, soil S content was highest in L+G (Fig. 7B). Overall, soil S content was highest in the 20–60 cm layer (Figure 7F and 7H), especially in the control at an N rate of 240 kg ha^{-1}.

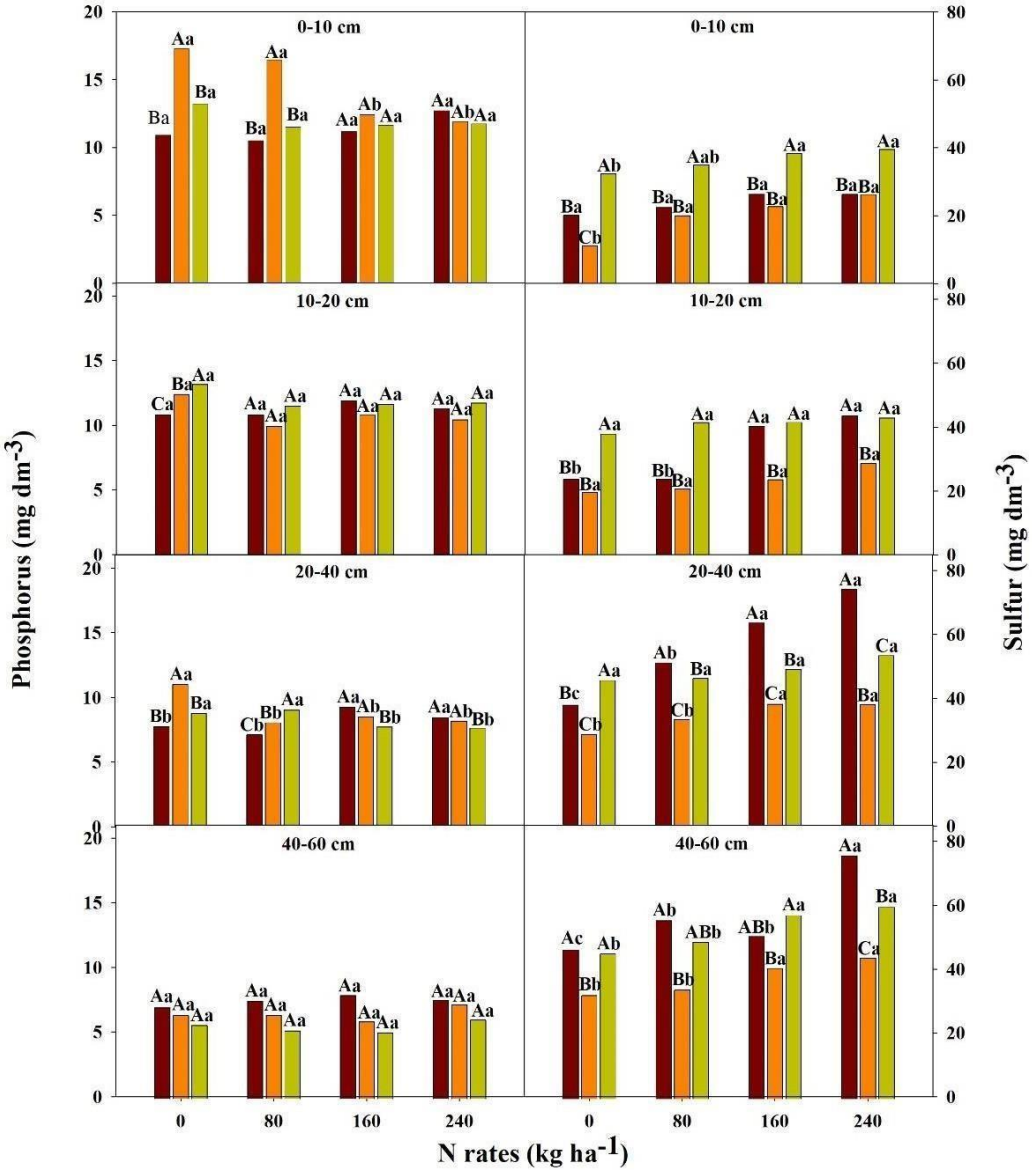

Fig. 7 - Phosphorus (P) and sulfur (S) contents in soil, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters compare soil correction management at each N dose.

Although L and L+G greatly increased soil Mg content in the 0–10 cm layer, only L+Gincreased soil Mg availability in the 10–20 and 20–40 cm layers. At high N rates, soil Mg content in the surface layer decreased in L and L+G (Fig.8A, 8C, 8E and 8G). Although soil Kcontent decreased in the surface layer and increased with depth in L+G compared with the control, this profile was lost in the presence of N fertilization (Fig. 8B and 8H).

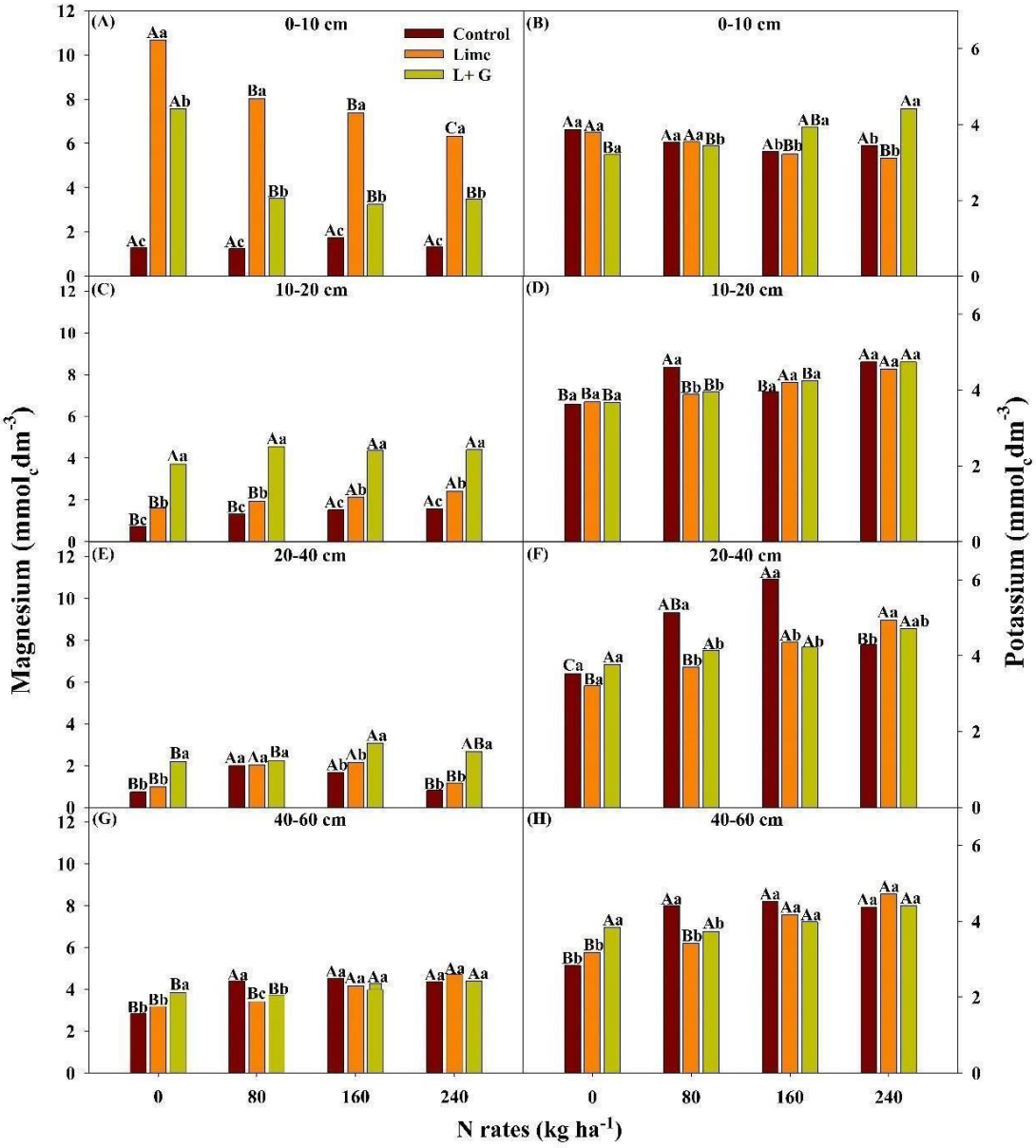

Fig. 8 - Magnesium (Mg) and potassium (K) contents in soil, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters compare soil correction management at each N dose.

Interestingly, L and L+G increased SOM content up to a depth of 40 cm, and the best combination for increasing SOM in the deepest soil layer was L+G at intermediate N rates (Fig.9). Synergistic effects of L+G and N were observed at N rates of up to 240 kg ha^{-1} in the 0–10cm layer and up to 160 kg ha^{-1} in the 10–20 cm layer. In the surface layer (0–10 cm), L+G hadno significant effect on SOM content compared with L, and the effects of the N rate were greatest in L+G. In deeper layers (> 40 cm), L reduced SOM content compared with the control(Fig. 9D), whereas L+G mitigated this effect.

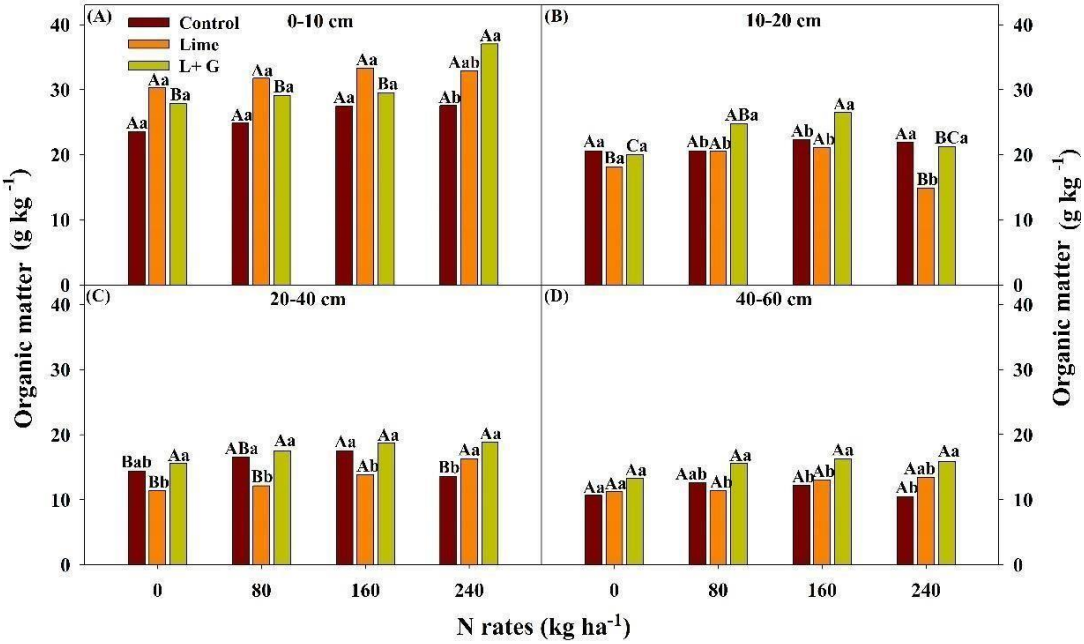

Fig. 9 - Organic matter contents in soil, depending on soil correction and nitrogen fertilization. Capital letters compare N doses in each soil correction management. Lowercase letters comparesoil correction management at each N dose.

2.4 Discussion

2.4.1 Yield, soil fertility, and leaf nutrient content

Overall, the contents of nutrients in soybean leaves were not deficient, even in the control treatment. The average contents of macro- and micronutrients in soybean leaves were within the expected ranges (Malavolta, 1980; Marschner, 1995). By contrast, in maize leaves in the control treatment, P and Mg contents were below the optimal concentrations (2 g kg^{-1}; Malavolta, 1980). L or L+G combined with 160 kg ha^{-1} N resulted in adequate maize leaf P content (Table 2). Regardless of the N rate, only L resulted in

sufficient maize leaf Mg content(Table 2).

The effect of soil acidity alleviation by L+G on yield was greater for double-cropped maize than for soybean. This difference is at least partly attributable to the greater tolerance of soybean to relatively high soil Al saturation (Mascarenhas and Tanaka, 1995). It may also be associated with climatic conditions, as precipitation was 203 mm (2021) and 295 mm (2022) during the maize seasons but 763 mm (2021) and 1009 mm (2022) during the soybean seasons.Compared with the control and L, L+G increased soil Ca availability in deeper layers (Fig. 6), enabling greater crop root growth (Souza et al., 2023) and improved water acquisition under drought.

The N rates needed to reach maximum yields and improve soil fertility were lower in L+G than in L and the control due to the greater alleviation of acidity and increased availability of Ca and Mg in L+G. These results corroborate prior findings that liming improves the N-use efficiency of crops and reduces their dependence on fertilization (Souza et al., 2023). Maize yields were lowest in control regardless of the N fertilization rate, indicating that the improvement in soil conditions by L+G made soil reserves sufficient to meet the N demand for maize. L+G may have increased root growth or accelerated the decomposition of SOM, mainly from recently deposited residues. We anticipated that the response of yields to the application of lime and phosphogypsum would increase as the N rate increased due to soil acidification. For soybeans, acidity correction increased productivity relative to control. It is possible that in low acidic soil, N fertilization further reduces soybean nodulation, as reported by Cordeiro and Echer (2019). For maize, greater responses of yield to N fertilization were expected. We observed greater responses in amended soils and lower responses in acidic soils. The severe climate conditions during the two seasons (Fig. 1) likely limited the level of productivity such that the application of lime and phosphogypsum was sufficient to meet the nutritional demands of maize plants.

Alleviating soil acidity increases N absorption. Alves et al. (2021) recently found thatliming favors biological N fixation and improves N uptake by soybeans. In acidic soils, Al toxicity inhibits nodule development due to effects on both plants and rhizobia (Morón et al., 2005; Lin et al., 2012). Al impairs nodulation by inhibiting the synthesis of flavonoids and Nod factor expression (Hungria and Stacey, 1997). Furthermore, low pH induces oxidative stress and the production of reactive oxygen species and salicylic acid, which also negatively affects nodulation (Velikova et al., 2000).

L+G increased soybean P leaf content, which can be attributed to a decrease in phosphate adsorption as the pH of the soil surface layer increased (Penn and Camberato,

2019).Liming favors the mineralization of organic P in soil (Besen et al., 2024). Soil pH plays a key role in P availability, and in this study, L+G increased P availability, especially in the 10 –20 cm layer (Fig. 7C). In soil treated with, phosphogypsum and lime, the greater availability of hydroxyls in the soil solution reduces P sorption by increasing competition for adsorption sites (Sato and Comerford, 2005). Furthermore, in acidic tropical soils, variable charges predominate, and net negative charges increase P availability by reducing phosphate adsorption through repulsion mechanisms (Barrow, 1986). In addition to increasing pH, L+G increased theavailability of Ca, which plays a crucial role in binding SOM to inorganic colloids (Muneer andOades, 1989).

2.4.2 Soil aggregates

Lime and phosphogypsum amendment aid the formation of larger aggregates by supplying cations (Ca^{2+} and Mg^{2+}) that form cationic bridges and flocculate clays (Wuddivira and Camps-Roach, 2007). Liming increases CEC in highly weathered soils, where CEC depends on soil pH (Costa et al., 2021). In addition, the application of lime and phosphogypsumincreases organic matter input in soil by promoting root growth (Barcelos et al., 2022) and increasing the supply of residues from the aerial portion (straw) of the plant. A previous study (Chan and Hennan, 1993) observed a direct correlation between the ASI and the lime application rate, suggesting that liming results in the formation of new Ca bonds. Liming provides Ca^{2+} and Mg^{2+}, which act as soil-binding agents by forming cationic bridges between clay particles and SOM (Tisdall and Oades, 1982; Castro et al., 2017). Therefore, the increasesin soil Ca content (Fig. 6) and soil Mg content (Fig. 8) in L+G, especially in the absence of N fertilization, may be directly related to the increases in the ASI (Fig. 3) and aggregate size (Figs.4 and 5).

Improvements in soil structure (i.e., increases in GMD and WAD) are also important for increasing soil water storage capacity (Bagnall, et al., 2022) and, consequently, drought tolerance and yields under drought. The differences in the responses of the soil stability indicators (i.e., ASI, WAD, and GMD) to the treatments reflect differences in what these indicators measure. For instance, ASI, which measures the proportion of aggregates larger than 0.25 mm, reflects the physical and chemical effects of the treatments on aggregate stabilization(Fig. 3). An increase in GMD from 0.5 mm to 1.5 mm (Fig. 4) indicates root effects. N fertilization increased WAD, especially in L+G. The positive effect of N fertilization on soil aggregation is consistent with the results of Wang

et al. (2018), who found that N fertilization increased the proportion of large macroaggregates (>2 mm) and the N content of macroaggregates (>2 and 1–2 mm). Wang et al. (2018) attributed these changes to increases inroot biomass due to improved plant nutrition and increased fungal biomass in macroaggregates. The biotic parameters of root and fungal biomass are crucial for the formation of macroaggregates (Bronick and Lal, 2005).

The positive effects of liming on the characteristics of soil aggregates, especially WADand GMD, were expected due to the roles of Ca and Mg in soil aggregation. However, Neto et al. (2021) foundlittle effect of lime and phosphogypsum application on soil physical propertiesin a no-till system. It is possible that strong interactions between SOM and minerals that act asbinding agents facilitate soil aggregation even in acidic soils (Azevedo and Bonumá, 2004). Incontrast to the results of Neto et al. (2021), L+G increased soil aggregation in the present study,suggesting an increase in C content due to the physical protection of SOM (Rowley et al., 2018).L+G increased SOM content, including in subsurface layers (Fig. 10), consistent with previousreports that the combination of lime and phosphogypsum promotes subsoil fertility, and increases root growth deeper in the soil profile (Pivetta et al., 2019; Souza et al., 2023), and thus increasessubsurface C inputs (Inagaki et al., 2017; Jones et al., 2018). Most studies of the impacts of soilacidity alleviation and fertilization on soil attributes have examined surface layers (<20 cm), and there is little information in the literature on subsoil layers. In our study, we observed improvements in soil physical attributes at depths of up to 60 cm.

The significant influence of liming on pH at low N rates (Fig. 6) may have been due to the use of N fertilizer based on NH_4^+, which has the potential to acidify the soil (Garbuio etal., 2011), especially on the surface layer (Fig. 6A). Thus, at higher N rates, soil acidification reduced the effectiveness of liming in improving soil aggregation. N fertilization can reduce, increase, or have no effect on the stability of soil aggregates (Blanco-Canqui et al., 2014). Our results suggest that the use of ammoniacal N fertilizers may reduce aggregate formation and stability by dispersing colloids and secondary soil particles (Haynes and Naidu, 1998) and reducing microbial biomass (Biederbeck et al., 1996). Others have suggested that N fertilizationcauses aggregate breakdown (Fonte et al., 2009). Soil mobilization as a result of frequent disturbances by mechanical implements reduces aggregate stability, and N fertilization can cause aggregate dispersion and increase macroaggregate cycling by degrading organic bindingagents within soil aggregates (Guo et al., 2022).

2.5 Conclusions

The impact of soil acidity alleviation by lime and phosphogypsum on crop productivity was greater in environments with water restrictions and lower N input. Although the effects of soil correction and N fertilization on soil aggregation and fertility were most evident in the surface layer, improvements were observed at soil depths of up to 60 cm, mainly in L+G.

L+G improved soil aggregation directly based on the roles of Ca and Mg as cation bridges. L+G may also have had indirect effects on soil aggregation, such as improving root development by decreasing Al levels. These effects improved crop productivity by increasing soil fertility and soil physical quality (i.e., soil aggregation and C storage). For maize, only L+G provided the maximum yield. L+G also reduced the need for N fertilization and increased soil quality and crop drought tolerance.

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CHAPTER 3

SOIL CARBON MINERALIZATION: A CLOSER LOOK ONTHE ROLE OF AGGREGATES, pH ALLEVIATION AND N APPLICATION[3]

The paper will be submitted to the European Journal of Soil Science.

3.1 Introduction

Soil amendments, such as gypsum and lime, play a crucial role in enhancing the qualityof tropical soils by improving its structure, fertility, carbon (C) and nitrogen (N) mineralization (Vazquez et al., 2019), thereby influencing crop yields and overall profitability (Castro et al., 2017; Souza et al., 2023). An important indicator of soil quality is the organic matter content (Franzluebbers, 2002), which impacts various aspects of soil quality, including soil aggregation(Ferrari Neto et al., 2021) and soil microbial activity (Cordeiro et al., 2021a). This, in turn, supports sustainable food production and maintains overall environmental quality (Verma et al., 2010; Franzluebbers, 2013).

Therefore, soil quality is intricately linked to C content, which is affected by practicessuch as soil acidity alleviation, N application and no-tillage (Sarto et al., 2020; Cooper et al., 2021; Barcelos et al., 2022). The availability of N and higher microbial populations in the rhizosphere significantly contribute to the accumulation of organic C in the soil (Sarto et al., 2020; Barcelos et al., 2022). Grass residues with a high C:N ratio have a long persistence, favoring soil organic matter (SOM) accumulation and reducing mineralization (Teixeira et al., 2014). Thus, no-till with balanced inputs of C and N could increase C sequestration and reduce the rate of C mineralization (He et al., 2023). Consequently, the choice of crop rotation and cropping system can impact organic C accumulation, affecting the soil's biological activity andaggregation.

[3] Chapter written following the guidelines of the European Journal of Soil Science.

Additionally, soil organic C is directly associated with total N through strong biological bounds (Cleveland and Liptzin, 2007). Changes in organic C content affect soil totalN, predominantly in its organic fraction (Pringle et al., 2014). Nitrogen fertilizer addition influences soil organic C accumulation, depending on the C source introduced by agricultural production, residues, roots, and the stimulation of C decomposition (Dalal et al., 2011; Geisseler; Scow, 2014; Stewart et al., 2017).

N fertilization can affect N pools in the soil, affecting C mineralization (Moran et al.,2005; Luo et al, 2019; Jiang et al., 2023) by accelerating the decomposition of light C fractionswhile enhancing the stabilization of soil C compounds into heavy fractions (Neff et al., 2002;Zhong et al., 2015;). The rhizosphere's C content is increased by N application due to a higherrhizodeposition and root biomass (Luo et al., 2019). The incorporation of this C by N fertilization primarily occurs in large aggregates (0.25-2.00 mm) and the humic fraction of thesoil (Luo et al, 2019). As the N cycle quickens, the ability of ecosystems to retain N becomes acrucial ecosystem service (Castellano et al., 2012). The joint incorporation of crop residues withan appropriate rate of N fertilizer significantly boosts soil C accumulation (Moran et al., 2005).On the other hand, N addition in tropical forest soils results in slower C cycling and increased sequestration, accompanied by elevated N2O emissions (Tian et al., 2019; Zheng et al., 2022).

The accumulation of crop residues on the soil surface provides C and N inputs, creatinga zone with a high potential for soil biological activity, as observed in soil C and N mineralization under standard laboratory conditions (Franzluebbers et al., 2018). However, enhanced aggregation can also limit C and N mineralization, since C can be protected in stable aggregates (Cooper et al., 2021), which inhibits mineralization, promoting carbon retention in the soil (Cambardella and Elliott, 1992). Carbon can be encapsulated in aggregates, shielded from microbial activity (Tisdall and Oades, 1982), or adsorbed on mineral surfaces (Janzen, 2015). Although intrinsically susceptible to deterioration, the mineral matrix can protect soil organic C (Wissing et al., 2014).

Carbon and N dynamics are complex in the soil environment where different practicessuch as lime, gypsum, and nitrogen application can lead to complex reactions, which can or cannot resultnot in soil C accumulation (Barcelos et al., 2021; De Souza et al., 2023). Therefore, we hypothesize that C mineralization is lower with N applications in association with soil acidity alleviation with lime and gypsum applications. To test this hypothesis, we evaluated C stability, the potential of C mineralization, and C in the

microbial biomass in a no-till system when gypsum, lime, and N were applied.

3.2 Material and Methods

3.2.1 Characterization of the studied area

A long-term field experiment was used as a framework to assess the effects of combined soil practices (N fertilization, lime, and phosphogypsum application) on C and N mineralization. The no-till field trial in Botucatu, State of São Paulo, Brazil, situated at an elevation of 780 meters asl, 22º 49´ 50" S, 48º 25´ 38" W. The area experiences a mesothermalclimate characterized by wet summers and dry winters. The average annual temperature is 20.7°C, with monthly highs averaging 26.1°C and lows at 15.3°C. Rainfall averages 1696 mm annually. The soil is classified as Typic Haplorthox (Soil Survey Staff, 2014). Prior to the study,soil fertility attributes were analyzed, and the results are shown in Table 1. The experiment wasinitiated in October 2016 to assess the impact of lime, lime combined with phosphogypsum, and N fertilization on soybean (*Glycine max*) double-cropped with maize (*Zea mays*) intercropped with Guinea grass (*Megathyrsus maximum*).

Table 1. Soil chemical and physical attributes and particle size distribution before the implementation of the experiment. August 2016.

Layer	pH	OC	P_{resin}	S	Al	K	Ca	Mg	CEC	Sand	Clay
	$CaCl_2$	g dm^{-3}	mg dm^{-3}				$mmol_c$ dm^{-3}			g kg^{-1}	
0-10 cm	4.3	10.2	12.8	4.8	9.3	2.1	11.9	11.8	78.2	540	343
10-20 cm	4.2	8.1	10.1	4.7	9.0	1.1	9.4	7.5	65.6	520	368

OC = organic carbon; CEC = cation exchange capacity.

3.2.2 Experimental design

The experimental design followed the same as established in the field, which consistedof a 3x2 factorial in complete randomized blocks with four replications. The treatments were acombination of control (C: without application of lime and gypsum), application of lime (L), and lime + gypsum (L+G), in addition to two N rates (0 and 240 kg ha^{-1}) applied annually to maize. Soybeans were grown in spring/summer and double-cropped maize in fall/winter each year. Lime was applied in a dose of 2.9 Mg ha^{-1} and

gypsum was applied in a dose of 2.0 Mg ha^{-1}.

3.2.3 Soil sampling and analysis

Three random sub-samples were collected per plot at depth from 0 to 20 cm using a stainless shovel in 2022 and combined into one sample per plot. The soil was passed through a5-mm mesh to remove stones, roots, and macrofauna and ensure homogeneity. For soil aggregate analysis, soil monoliths were collected, gently divided into smaller fractions, and passed through an 8-mm screen before drying at 50°C for 48 hours. These samples were shipped to Raleigh, North Carolina for further analysis. The soil was separated into four classes of aggregates according to Garland et al. (2017), namely: sand and iron concretions (> 2000 µm),macro-aggregates (< 2000 µm and > 250 µm), micro-aggregates (53 µm) and silt and clay (< 53 µm).

The soil from sieved samples (2 mm) was ground into powder to determine total organic carbon by dry combustion at 1350°C (Leco CNS-2000, St. Joseph, Michigan). Mineralizable C and particulate organic matter determinations followed Franzluebbers and Brock (2007). Inorganic N (ammonium [NH_4^+ -N] + nitrite [NO_2 -N] + nitrate [NO_3^- -N]) was determined from the filtered extract of a 10-g subsample of oven-dried (55°C, 48 hours) and sieved (<2 mm) soil stirred with 20 mL of 2M KCl for 30 minutes using salicylate-nitroprusside and hydrazine autoanalyzer techniques (Bundy and Meisinger, 1994).

3.2.4 C and N mineralization assay

To assess potential C and N mineralization and C in the microbial biomass, the soil was oven-dried at 55°C for 3 days. Oven drying was chosen as the standard procedure to facilitate rapid drying and to be consistent with the previous experimental procedure (Franzluebbers and Arshad, 1997). The dry soil was moistened to 50% pore space at the beginning of aerobic incubation. The soil was placed in a jar with a flask containing 10 ml of NaOH to retain the released CO_2 and a water flask to maintain moisture. The jars were sealed and dark-incubated at 25±1°C for up to 24 days. Alkali traps were replaced on day 3. Carbon mineralization during the periods of 0-3 and 3-24 days was determined as total CO_2 evolved byalkali titration with 1M HCl (Anderson, 1982). Inorganic N (NO_3^- –N NO_2 –N and $NH4^+$ –N) was determined in dry soil sieved to <2 mm (10 g soil, 20 ml 2M KCl)

for each soil aggregate class and depth and disturbance regime at 0 and 24 days of incubation (55°C, 48 h) using hydrazine and salicylate autoanalyzer techniques (Bundy and Meisinger, 1994). Soil organic Nand C were determined in dry (55°C, 48 h) and ground subsample using dry combustion.

3.2.5 Statistical analysis

Data were tested for homogeneity of variance and normality and were subjected to ANOVA, and then means were compared by the Tukey ($P < 0.05$) test using SAS version 9.2 (SAS Institute Inc 2009). A principal component analysis (PCA) was performed to reduce data, visualize, and explore the relation between the variables. The PCA was performed using R (RCore Team, 2021).

3.3 Results

3.3.1 Soil test biological activity

In general, soils without nitrogen application showed higher soil test biological activitythan the soils with N (Fig. 1). This is clearly expressed for the average of all sieves (Fig. 1F), where the control treatment without N showed 25.4% higher soil biological activity than the control treatment with N. Considering the average of all aggregates size, the soil amendments affected soil test biological activity only in soils without N, in which lime application decreasedsoil biological activity in 21.1% compared to the control (Fig. 1F). With N application, there isno effect of soil amendments on soil biological activity, which showed an average of 70.6 mg C kg^{-1} day^{-1} (Fig. 1F).

Regarding N effects on soil biological activity considering aggregates size, there was no N effect in aggregates larger than 2 mm (Fig. 1A) and smaller than 0.25 mm (Fig. 1E). In mesoaggregates (i.e., smaller than 2 mm and larger than 0.5 mm) soils without N showed up to47.6% higher soil test biological activity when compared to soils with N (Fig. 1C).

Soil amendments resulted in no response to soil biological activity considering different aggregate sizes, with an exception for aggregate size from 0.5 mm to 0.25 mm (Fig. 1D). On this class of aggregates, when N is applied, the combined application of lime and gypsum (L+G) resulted in 33.6% higher soil biological activity than control (C) and

only lime (L) application.

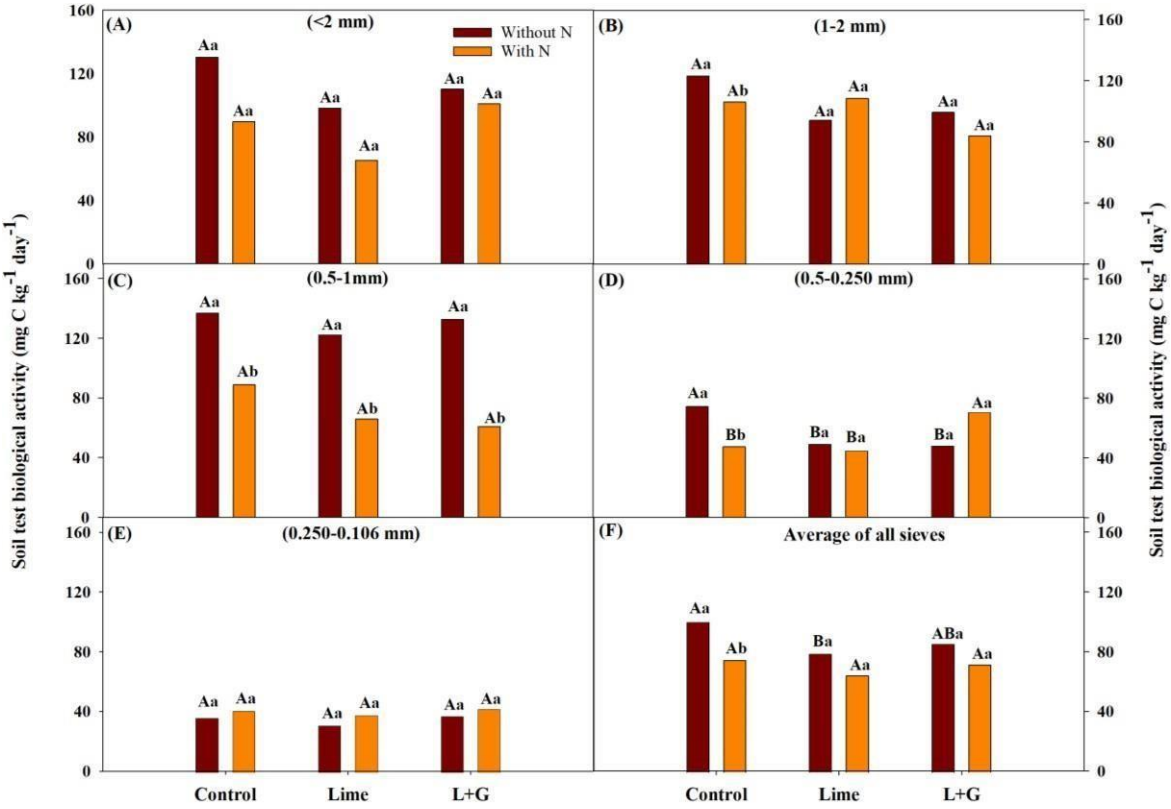

Fig. 1. Soil test biological activity as a function of soil amendments (lime – L and lime + gypsum – L+G) and nitrogen fertilization in different aggregates sizes (from A to E) and the average of all aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

3.3.2 Basal soil respiration

In general, considering the average size of all aggregates, nitrogen, lime, and gypsum application did not affect basal soil respiration, which was on average 8.23 mg kg^{-1} day^{-1} of C (Fig. 2F). Regarding aggregate size, only aggregates larger than 2 mm and smaller than 0.25mm were responsive to soil amendments (Figs. 2A, 2E). In addition, those differences in these aggregates classes were observed only in the absence of nitrogen.

In aggregates larger than 2 mm, lime, and lime+gypsum decreased by 43.8% basal soilrespiration compared to the control (Fig. 2A). On the other hand, in aggregates smaller than 0.25 mm, lime application increased soil basal respiration by 48.1% compared to control and lime+gypsum application (Fig. 2E).

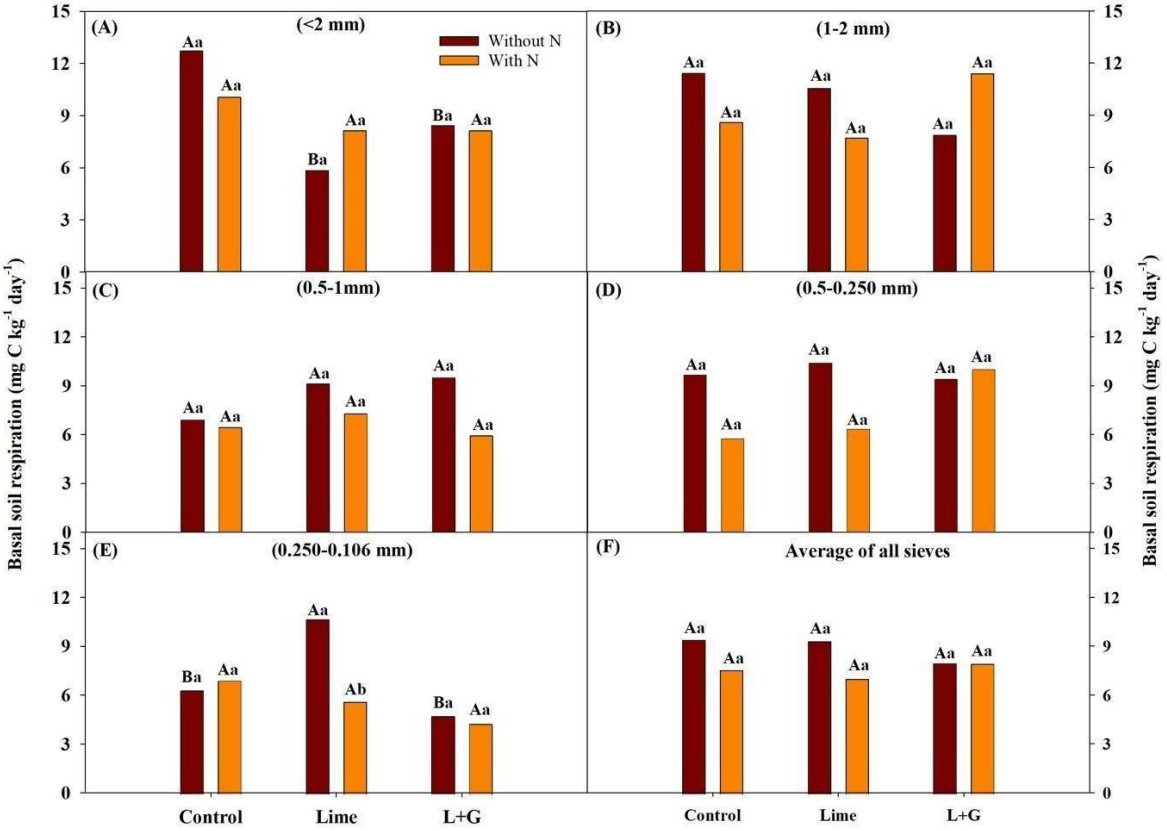

Fig. 2. Basal soil respiration as a function of soil amendments (lime – L and lime + gypsum – L+G) and nitrogen fertilization in different aggregates sizes (from A to E) and the average of all aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

3.3.3 Soil carbon mineralization

In general, considering the average of all aggregate sizes, nitrogen and soil amendments application did not affect net carbon mineralization, which was on average 252.7mg C kg^{-1} after 24 days of incubation (Fig. 3F).

Regarding aggregate size, nitrogen application resulted in lower net C mineralization, especially for mesoaggregates (up to 1 mm, Figs. 3C and 3D) and microaggregates (0.25 mm – 0.106 mm; Fig. 3E), and under lime + gypsum (Fig. 3C) and limemanagements (Fig. 3D and 3E).

Concerning lime and gypsum application, only aggregates larger than 2 mm and smaller than 0.25 mm were responsive, and just under nitrogen absence (Figs. 3A, 3E). In aggregates larger than 2 mm, lime, and lime+gypsum decreased by 36.7% net C mineralization compared with the control (Fig. 3A). Conversely, in aggregates smaller than 0.25 mm, lime application increased net C mineralization by 39.2% compared to

control and lime+gypsumapplication (Fig. 3E).

Overall, C mineralization was higher under N absence and in aggregates larger than 0.5 mm (Fig. 3A) and lower in microaggregates (0.25 – 0.106 mm) with N, lime, and gypsumapplication (Fig. 3E).

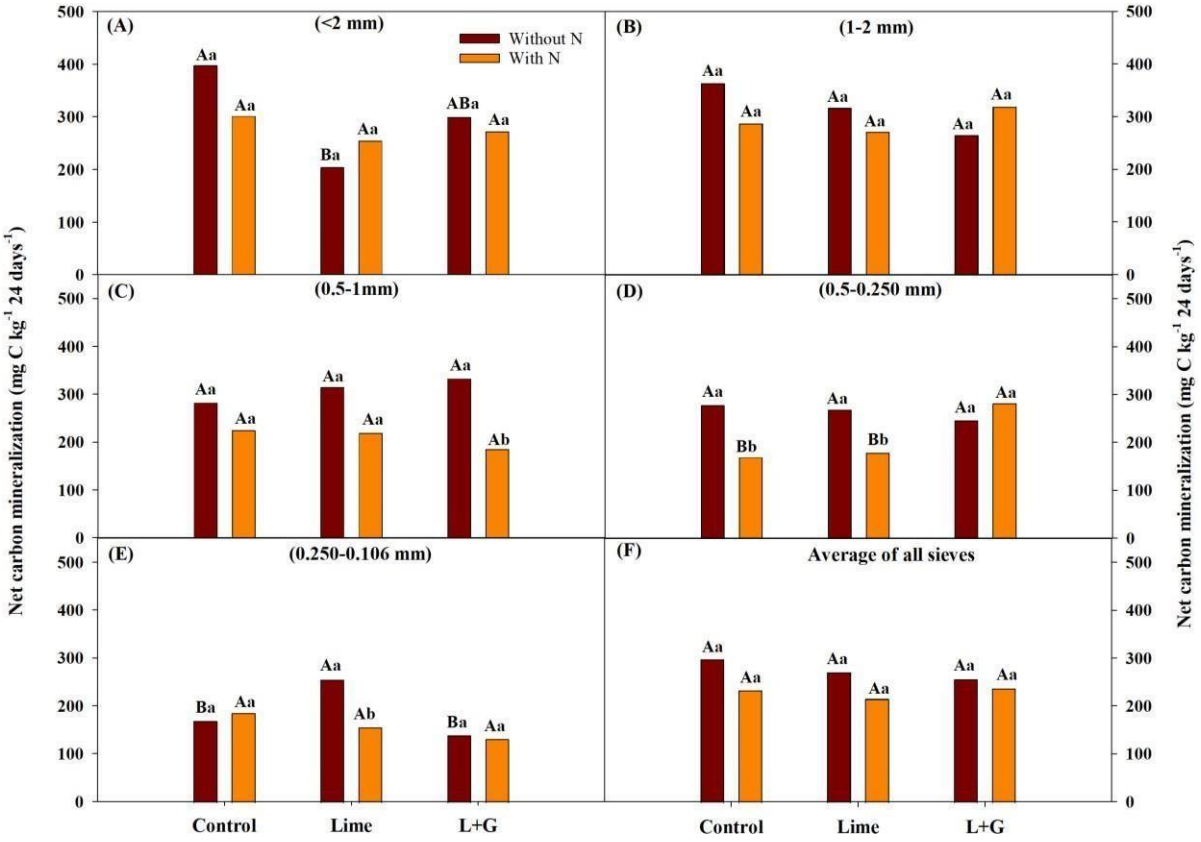

Fig. 3. Net carbon mineralization as a function of soil amendments (lime – L and lime + gypsum
– L+G) and nitrogen fertilization in different aggregate sizes (from A to E) and the average ofall aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

3.3.4 Soil Nitrogen Mineralization

The impact of N addition on net N mineralization varied depending on the soil management and aggregate size (Fig. 4). Considering the average of all aggregate sizes, the addition of N did not affect net mineralization (Fig. 4F). Regarding soil management, under N deficiency, lime + gypsum resulted in 19.9% higher net N mineralization than the control and sole lime application (Fig. 4F). With N application there was no difference between the soil amendments.

Clear effects of soil amendments and nitrogen application were observed on the aggregate larger than 2 mm, in which the highest net N mineralization rates were observed (Fig. 4A). In addition, contrasting responses were found with and without N application on this aggregate size. Without N, net N mineralization was higher under lime + gypsum management. On the other hand, when N was applied, higher net N mineralization was recorded in the control.

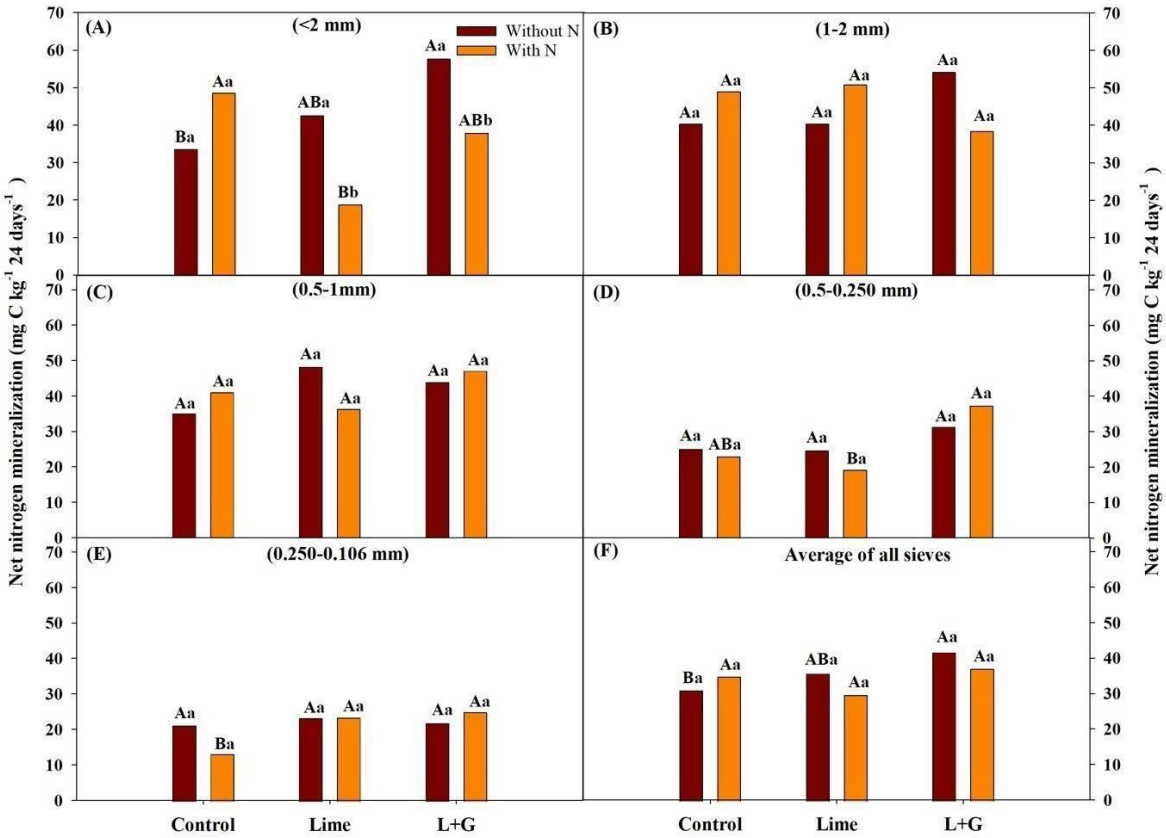

Fig. 4. Net nitrogen mineralization as a function of soil amendments (lime – L and lime + gypsum – L+G) and nitrogen fertilization in different aggregate sizes (from A to E) and the average of all aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

In general, on average of all aggregate sizes, N, lime, and gypsum application did not affect the soil residual inorganic N contents, which was on average 12.94 mg kg^{-1} (Fig. 5F). On the other hand, considering different aggregate sizes, residual inorganic N was highest in aggregates < 2mm under lime and N application. The soil management with N and lime also showed a higher residual inorganic N for the aggregates from 0.5 to 0.25 mm (Fig. 5D).

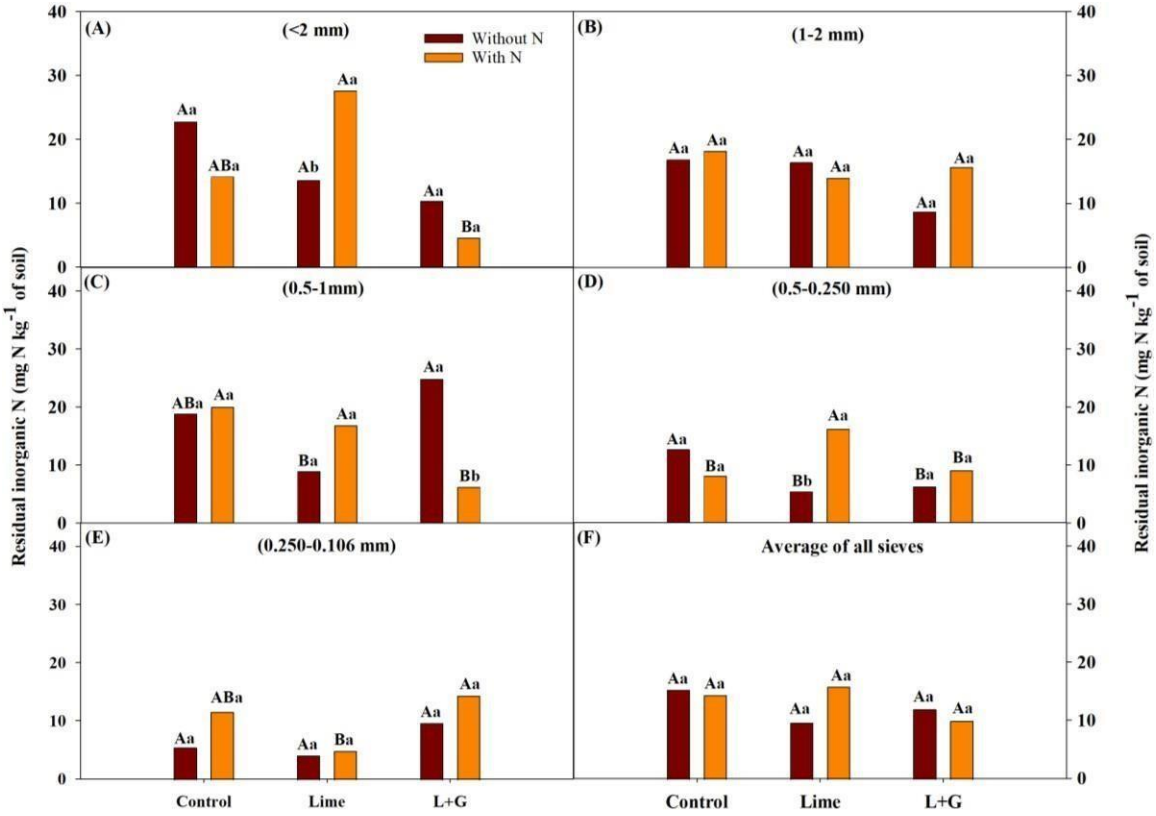

Fig. 5. Residual inorganic N as a function of soil amendments (lime – L and lime + gypsum – L+G) and nitrogen fertilization in different aggregate sizes (from A to E) and the average of all aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

Overall, considering the average of all aggregate sizes, nitrogen and soil amendment application did not affect residual soil nitrate contents, which were on average 0.83 mg kg^{-1} (Fig.6F).

However, significant differences were found considering the aggregates size. Generally, higher residual soil nitrate levels are observed with N application across most treatments and aggregate sizes (Fig. 6). Regarding soil management, higher residual nitrate contents were observed in the treatments with lime, especially aggregates larger than 0.25 mm (Figs. 6A, 6B, 6C, and 6D). In microaggregates (0.25 – 0.106 mm), the soil management with lime, gypsum, and nitrogen showed higher residual soil nitrate (Fig. 6E).

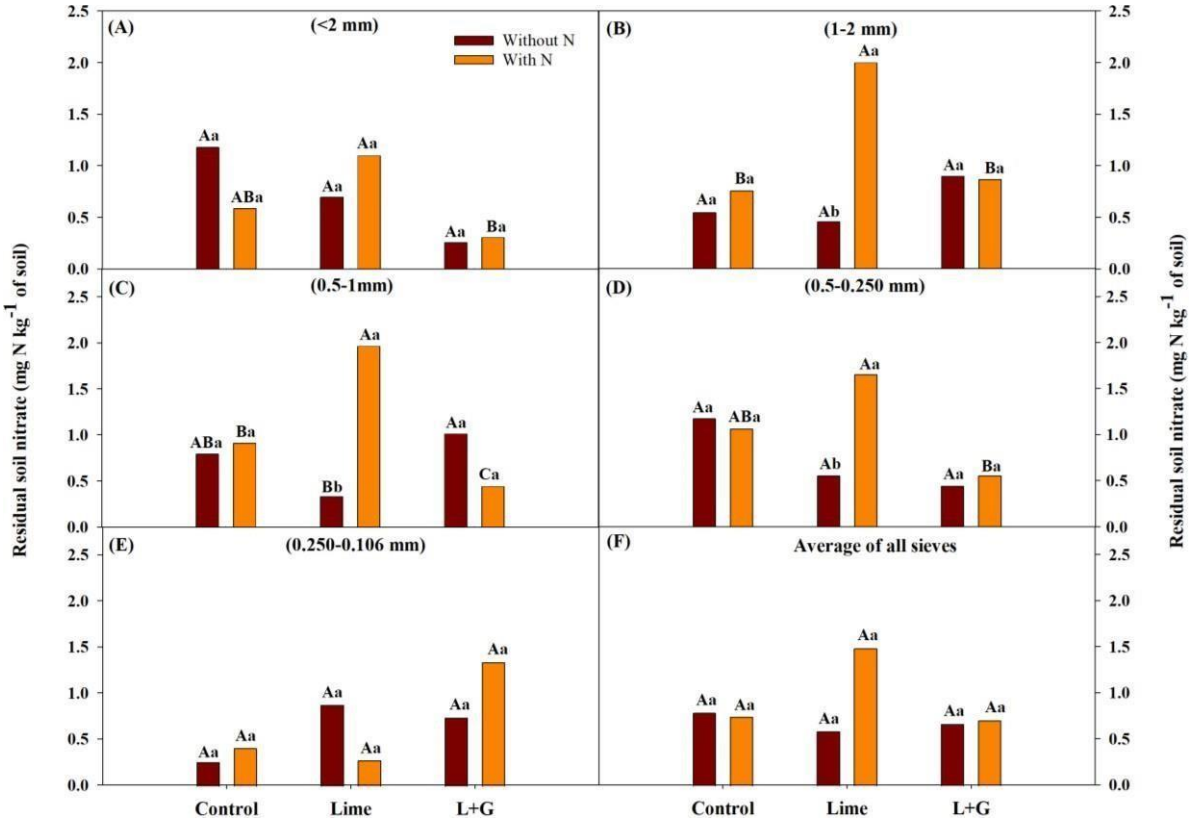

Fig. 6. Residual soil nitrate as a function of soil amendments (lime – L and lime + gypsum – L+G) and nitrogen fertilization in different aggregate sizes (from A to E) and the average of all aggregates (F). Uppercase letters compare soil correction amendment at each N dose. Lowercase letters compare the N rate within each soil amendment.

3.3.5 Principal Component Analysis

The principal component analysis showed two principal components (PC) which explained 64.4% of data variability. PC1 comprised 34.8% of data variability. PC2 explained 29.5% of data variability and revealed a contrast between RSN, RIN, and RSA variables vectors and net N mineralization, net C mineralization, basal soil respiration, and soiltest biological activity variables vectors.

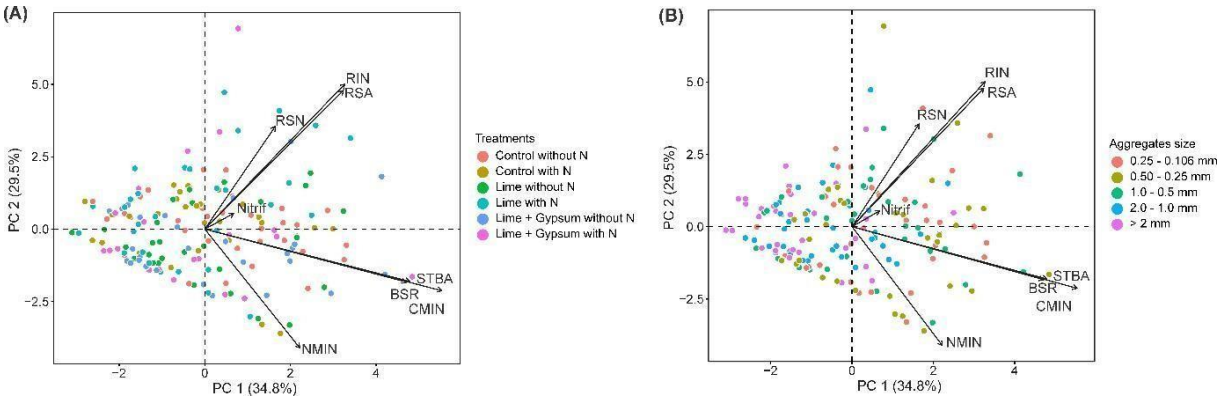

Fig. 7. Principal component analysis of soil attributes under C and N mineralization assays. STBA: soil-test biological activity; BSR: basal soil respiration; RSA: residual soil ammonium; RSN: residual soil nitrate; RIN = residual inorganic; NMIN24 = net N mineralization; Nitrif = apparent net nitrification.

3.4 Discussion

Soil basal respiration and C and N mineralization can express complex patterns underdifferent soil amendments and N rates. Some studies suggest that N application can increase basal soil respiration and improve soil quality (Ding et al., 2010; Chakraborty et al., 2011), while other studies show it may have a stimulatory or inhibitory effect depending on factors such as labile organic C concentration and land-use (Ni et al., 2012). Sometimes, it may have no significant effect (Chung and Chang, 2012) or even decrease respiration rates when combined with liming (Johnson et al., 2005).

Our findings indicate that, in general, different patterns were observed in C mineralization when nitrogen was applied alone, lime + gypsum was applied without N, and lime + gypsum was applied with N. In low fertility soils (e.g., control treatment) microbial mining of soil organic matter can intensify, potentially surpassing the formation of new C through the humification of fresh carbon, which leads to an imbalance and a net destruction of C (i.e., priming effect; Bingeman et al., 1953). Our data corroborates these previous findings since without lime, gypsum, or nitrogen, the control soils exhibited higher basal soil respirationrate and increased carbon mineralization, which could be a response to the soil fertility limitation that stresses the soil microbial community (Griffiths et al., 2012). Conversely, when nutrients are available, microbial mining of C is expected to decrease, allowing greater sequestration of nutrients within SOM (Fontaine et al., 2011; Barcelos et al., 2021). Higher C immobilization in the microbial biomass was achieved due

to gypsum, lime, and nitrogen application in highly weathered soils, highlighting that nutrient availability was more related togreater microbial C use efficiency than just soil acidity alleviation, as showed by Barcelos et al. (2021). Similarly, our results showed a nominal decrease in soil basal respiration and carbon mineralization comparing all treatments without N and with N (Fig. 2). However, N fertilizationwas reported to amplify the priming effect (Fontaine et al., 2011; Chen et al., 2014). For instance, the application of inorganic N fertilizers to agricultural systems with high C:N ratio residues (e.g., gramineous plants, Vahdat et al., 2011; Rosolem et al., 2017) may promote the extraction of C or N from SOM by microorganisms (Chen et al., 2014). Consequently, native SOM decomposition may be increased, enhancing C mineralization rates and CO2 emissions (Chen et al. 2014). In our experiment, acidity alleviation with lime + gypsum decreased the difference between treatments with and without N, probably promoting a negative priming effect. In fact, the application of limestone, gypsum, and N in tropical soils can increase net C in soils (da Costa et al., 2021; Barcelos et al., 2022). Higher humin contents were found in thesesoils treated with lime, phosphogypsum, and N (data not shown). In fact, studies showed that liming can induce the desorption of humic substances from mineral surfaces. This C fraction isoften considered relatively recalcitrant and can contribute to long-term C storage (Garbuio et al., 2011).

Higher respiration under lime and lime + gypsum treatments (Fig. 2) was expected since, in general, lime application increases nitrification due to the increased pH (Yao et al., 2011). However, the effects of N fertilization on soil respiration mainly depended on the concentration of easily decomposed organic carbon in the soil (Ding et al., 2010). Therefore, Nfertilization could decrease basal soil respiration when N is released from soil organic carbon decomposition. Liming can also potentially decrease C mineralization, even if mineral N is added (Wachendorf et al., 2015). Many processes can control C mineralization rate in limed soils. For instance, liming can improve plant growth, thus increasing the C-input to soil (Silva et al., 2023). However, higher C-inputs do not mean higher net C in the soil (Kuzyakov 2010; Fontaine et al., 2011). In fact, our data showed that lime + gypsum associated with nitrogen enhanced the soil biological activity (Fig. 1), which favored C mineralization (Fig. 3). Consequently, the quality of remaining C in the no-till system could play an important role. Nitrogen availability affects carbon mineralization by influencing soil C cycling and storage, altering microbial activity, with diverse effects depending on soil type and C:N ratios (Nave etal., 2009; Averill et al., 2018; Cheng et al., 2020; Ali et al., 2020). However, in the long term, net C mineralization can

decrease over time, and it could be an effect of labile soil carbon depletion in soils. In fact, Magill and Aber (2000) showed that N immobilization was higher insoils incubated with more labile C sources (e.g., glucose) than those incubated with more complex carbon compounds (e.g., humic acid). Conversely, covalent bonds of C with Ca can increase the stability of organic matter (Muneer and Oades, 1989). In mineral soils, lime + gypsum can influence soil structure by flocculating and cementing soil aggregates due to the presence of Ca ions, protecting organic matter from decomposition (Baldock and Skjemstad, 2000).

Soil aggregates with different sizes create heterogeneous habitat structures, which causes variations in biological, chemical, and physical soil properties (e.g., nutrient availability,oxygen concentration, water content and microbial community; Bipfubusa et al., 2008; Wang et al., 2022). These variations can affect C and N mineralization. In fact, our data showed that smaller aggregates were more responsive to lime and lime + gypsum. This is clearly expressedin the principal component analysis (Fig. 7), in which smaller aggregates (i.e., 0.25 mm – 0.106mm) were found more on the left, in the direction of the soil-test biological activity, C mineralization, and basal soil respiration variables vectors. Many studies have shown that aggregate size can impact carbon mineralization rate, with smaller aggregates generally exhibiting greater cumulative levels (Tian et al., 2015; Zhang et al., 2016), and the highest mineralization rate found in 1-2 mm aggregates (Huang et al., 2019). This is also important in no-till systems where soil structure is improved, and a larger proportion of macroaggregates with relatively high carbon mineralization can contribute to high C stocks (Dong et al., 2023).

3.5 Conclusions

The combined application of lime + gypsum and nitrogen produced different effects on soil C and N mineralization when compared to individual treatments, showing stronger effects in smaller aggregates. Nitrogen deficiency significantly impacted microbial activity, increasing biological activity, soil respiration, and carbon mineralization, underscoring the importance of this essential nutrient in regulating carbon and nitrogen cycles. Aggregates fromdifferent sizes were differently affected by lime + gypsum application with and without N, being aggregates smaller than 0.5 mm more responsive. Nitrogen fertilization associated with lime and gypsum application results in higher basal soil respiration and C mineralization in aggregates smaller than 0.5 mm, what has been not reported before. Furthermore, we observedthat soil acidity alleviation and N

availability played a substantial role in C mineralization. These findings have important implications for sustainable soil acidity alleviation and effective fertilization practices, contributing to the optimization of agricultural techniques and the reduction of environmental impacts.

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CONCLUDING REMARKS

Soil acidity is one of the most important limitations to agricultural production worldwide. Although the primary goal of liming is to overcome soil acidity, it also impacts the chemical, biological, and physical properties of soils, affecting the entire system. Thus, the combination of lime, gypsum, and nitrogen fertilization was effective in improving soil quality and fertility, as well as increasing crop productivity. This combination resulted in a significant increase in soil carbon, especially in larger aggregates, which play a crucial role in carbon and nitrogen storage. Nitrogen fertilization proved essential for maximizing these benefits, confirming the importance of nitrogen in regulating soil carbon and nitrogen cycles.

Additionally, gypsum was fundamental in preventing the reduction of fulvic acid under liming and nitrogen fertilization conditions, thereby maintaining soil quality. The application of lime and gypsum improved soil aggregation. These improvements led to higher crop productivity, especially in water-limited environments, where the reduction in soil acidity had a significant impact.

Another important aspect is the reduction in soil basal respiration observed with the application of lime, gypsum, and nitrogen, indicating a decrease in biological activity and carbon loss. However, nitrogen deficiency increased carbon mineralization, especially in smaller aggregates. These results underline the importance of nitrogen in regulating carbon and nitrogen cycles, highlighting that the combined application of lime, gypsum, and nitrogen has distinct effects on carbon and nitrogen mineralization compared to individual treatments.

The results demonstrate that the combined application of lime, gypsum, and nitrogen fertilization not only improves soil fertility and physical quality but also contributes to optimizing agricultural techniques and reducing environmental impacts. Thus, we confirm our hypothesis that the combination of these treatments is essential for carbon and nitrogen accumulation in the soil.

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