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



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

**Botucatu
2024**

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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,
and my sister Maria Fernanda.*

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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 objective was 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 the accumulation 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 treatments were: 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 and a 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 applied together 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.

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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.

REFERENCES

- ALMEIDA, L. F. A. *et al.* Soybean yield response to nitrogen and sulfur fertilization in the United States: contribution of soil N and N fixation processes. **European Journal of Agronomy**, v. 145, p. 126791, 2023.
- ARAÚJO, L. G. *et al.* How does gypsum increase the organic carbon stock of an Oxisol profile under sugarcane? **Geoderma**, v. 343, p. 196-204, 2019.
- BARCELOS, J. P.; MARIANO, E.; JONES, D. L.; ROSOLEM, C. A. Topsoil and subsoil C and N turnover are affected by superficial lime and gypsum application in the short-term. **Soil Biology and Biochemistry**, v. 163, p. 108456, 2021.
- BARCELOS J.P.Q. *et al.* Soil acidity amelioration improves N and C cycles in the short term in a system with soybean followed by maize-Guinea grass intercropping. **Geoderma**, v. 421, p. 115909, 2022.
- BARTHES, B.; ROOSE, E. Aggregate stability as an indicator of soil susceptibility to runoff and erosion validation at several levels. **Catena**, Amsterdam, v. 47, p. 133–149, 2002.
- BODDEY R.M. *et al.* Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. **Global Change & Biology**, v.16, p.784–795, 2010.
- CAMBARDELLA C.A.; ELLIOTT E.T. Particulate soil organic-matter changes across a grassland cultivation sequence. **Soil Science Society America Journal**, v.56, p.777–783, 1992.
- CARVALHO, M.; CIARKOWSKA, K.; MIECHOWKA, A. Storage of persistent organic matter in temperate gypsum soils –Relevance of the parent material and vegetation cover. **Geoderma**, v. 435, p. 116522, 2023.
- CASTRO, G.S.A. *et al.* Management impacts on soil organic matter of tropical soils. **Vadose Zone Journal**, v. 2, p. 1-8, 2015.
- CHENG, X. *et al.* Assessing the effects of short-term *Spartina alterniflora* invasion on labile and recalcitrant C and N pools by means of soil fractionation and stable C and N isotopes. **Geoderma**, v. 145(3-4), p. 177-184, 2008.
- CHU, J. *et al.* Impacts of cropping systems on aggregates associated organic carbon and nitrogen in a semiarid highland agroecosystem. **Plos one**, v. 11(10), e. 0165018, 2016.
- CLEVELAND, C. C.; LIPTZIN, D. C:N:P stoichiometry in soil: is there a Redfield ratio for the microbial biomass? **Biogeochemistry**, Dordrecht, v. 85, p. 235–252, 2007.
- COOPER, H.V. *et al.* Long-term zero-tillage enhances the protection of soil carbon in tropical agriculture. **European Journal of Science**, v.72, p. 2477–2492, 2021.

- CORDEIRO, C. F. D. S. *et al.* Soil organic carbon stock is improved by cover crops in a tropical sandy soil. **Agronomy Journal**, v. 114(20), p. 1546-1556, 2022.
- CORDEIRO, C. F. S.; ECHER, F. R.; ARAUJO, F. F. Cover crops impact crops yields by improving microbiological activity and fertility in sandy soil. **Journal of Soil Science and Plant Nutrition**, v. 21(3), p. 1968-1977, 2021.
- COSTA, C. H. M. *et al.* Long-term effects of lime and phosphogypsum on soil carbon and nitrogen and physical attributes under tropical no-till. **Soil Science Society of America Journal**, v. 85(2), p. 328-339, 2021.
- CRUSCIOL, C.A.C. *et al.* Exploring the synergism between surface liming and nitrogen fertilization in no-till system. **Agronomy Journal**, v. 114, p. 1415-1430, 2022.
- DALAL, R. C. *et al.* Organic carbon and total nitrogen stocks in a Vertisol following 40 years of no-tillage, crop residue retention, and nitrogen fertilization. **Soil Tillage Research**, Amsterdam, v. 112, p. 133–139, 2011.
- FOG, K. The effect of added nitrogen on the rate of decomposition of organic matter. **Biological reviews**, v. 63(3), p.433-462, 1988.
- FOLONI, J. S. S.; ROSOLEM, C.A. Effect of mandium squeal and sulfate on cotton. I- Transport of cations anions in the soil. **Revista Brasileira e Ciência do Solo**, v. 30 (3), p. 425-432, 2006.
- FRANZLUEBBERS A.J. *et al.* Soil-test biological activity with the flush of CO₂: C and N characteristics of soils in corn production. **Soil Science Society of America Journal**, v. 82, p. 685–695, 2018.
- GEISSELER, D.; SCOW, K. M. Long-term effects of mineral fertilizers on soil microorganisms a review. **Soil Biology and Biochemistry**, Elmsford, v. 75, p. 54–63, 2014.
- HATTORI, T. Soil aggregates in microhabitats of microorganisms. **Reports of the Institute for Agricultural Research**, Sendai, v. 37, p. 23–36, 1988.
- JANZEN, H. H. Beyond carbon sequestration: soil as conduit of solar energy. **European Journal of Soil Science**, v. 66(1), p. 19-32, 2015.
- JONES, D.L. *et al.* 2018. Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. **Soil Biology and Biochemistry**, v. 117, p. 72-82,
- KELLEY, K.; STEVENSON, F. Forms and nature of organic n in soil nitrogen economy in tropical soils. London: **Springer**, p. 1–11, 1995.
- KÖGEL-KNABNER, I.; AMELUNG, W. Dynamics, Chemistry, and Preservation of Organic Matter in Soils. In: HOLLAND, H. D.; TUREKIAN, K. K. (ed.). Treatise

on geochemistry. 2nd. ed. Oxford: **Elsevier**, v. 12, p. 157-215, 2014.

KOU, B. *et al.* Differential responses of the properties of soil humic acid and fulvic acid to nitrogen addition in the North China Plain. **Environmental Research**, v. 214, p. 113980, 2022.

MATTILA, T. J. *et al.* How do farmers approach soil carbon sequestration? Lessons learned from 105 carbon-farming plans. **Soil and Tillage Research**, v. 215, p. 105204, 2022.

MOCKEVICIENE, I. *et al.* Influence of liming intensity on fractions of humified organic carbon in acid soil: A case study. **Sustainability**, v. 14 (9), p. 5297, 2022.

NDZELU, B. S. *et al.* Tillage effects on humus composition and humic acid structural characteristics in soil aggregate-size fractions. **Soil and Tillage Research**, v. 213, p. 105090, 2021.

NEFF, J. *et al.* Variable effects of nitrogen additions on the stability and turnover of soil carbon. **Nature**, v. 419, p. 915–917, 2002.

OVCHINNIKOVA, M. F. Changes in the content, composition, and properties of humic substances in particle-size fractions of soddy-podzolic soils under the impact of long-term drainage. **Eurasian Soil Science**, v. 51(6), p. 647-657, 2018.

PIVETTA, L. A. *et al.* Gypsum application, soil fertility and cotton root growth. **Bragantia**, v. 78(2), p. 264-273, 2019.

PROVE, B. G.; *et al.* Improvements in aggregation and infiltration characteristics of a krasnozem under maize with direct drill and stubble retention. **Australian Journal of Soil Research, Melbourne**, v. 28, p. 577–590, 1990.

PRINGLE, M. J. *et al.* The effect of pasture utilization rate on stocks of soil organic carbon and total nitrogen in a semi-arid tropical grassland. **Agriculture, Ecosystems & Environment**, v. 195, p. 83-90, 2014.

ROCHA, K. F. *et al.* Cover crops affect the partial nitrogen balance in a maize-forage cropping system. **Geoderma**, v. 360, p. 114000, 2020.

ROWLEY, M.C.; GRAND, S.; VERRECCHIA, É. P. Calcium-mediated stabilisation of soil organic carbon. **Biogeochemistry**, v. 137, p. 27–49, 2018.

SEXSTONE, A. J. *et al.* Direct measurement of oxygen profiles and denitrification rates in soil aggregates. **Soil Science Society of America Journal, Madison**, v. 49, p. 645–651, 1985.

SIDDIQUE, K. H. *et al.* Enhancing crop productivity for recarbonizing soil. **Soil and Tillage Research**, v. 235, p. 105863, 2024.

STEWART, C. E. *et al.* Seasonal switchgrass ecotype contributions to soil

organic carbon, deep soil microbial community composition and rhizodeposit uptake during an extreme drought. **Soil Biology and Biochemistry**, v. 112, p. 191-203, 2017.

TADINI, A. M. *et al.* Chemical characteristics of soil organic matter from integrated agricultural systems in southeastern Brazil. **European Journal of Soil Science**, v. 73(1), e13136, 2022.

TEIXEIRA, R.A. *et al.* Grasses and legumes as cover crop in no-tillage system in northeastern Pará Brazil. **Acta Amazônica**, v. 44, p.411–418, 2014.

TISDALL J.M.; OADES J. M. Organic matter and water-stable aggregates in soils. **European Journal of Soil Science**, v. 33, p.141–163, 1982.

VIRK, A. L. *et al.* Simultaneous effects of legume cultivation on carbon and nitrogen accumulation in soil. **Advances in agronomy**, v. 171, p. 75-110, 2022.

WALIA, M. K.; DICK, W. A. Gypsum and carbon amendments influence carbon fractions in two soils in Ohio, USA. **Plos one**, v. 18(4), e0283722, 2023.

WANG, X.; YOST, R. S.; LINQUIST, B. A. Soil aggregate size affects phosphorus desorption from highly weathered soils and plant growth. **Soil Science Society of America Journal**, Madison, v. 65, p. 139–146, 2001