

JÉSSICA PIGATTO DE QUEIROZ BARCELOS

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

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FUNÇÃO DA CALAGEM, GESSAGEM E ADUBAÇÃO NITROGENADA**

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Orientador: Prof. Dr. Ciro Antonio Rosolem

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

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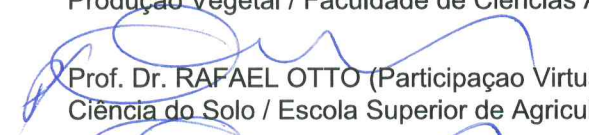
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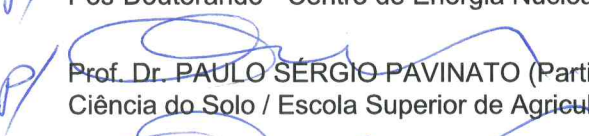
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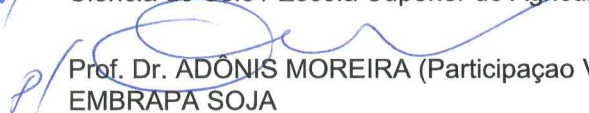
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EMBRAPA SOJA

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Aos meus pais Selma e Valter,

Ao meu irmão Gabriel,

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RESUMO

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

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

ABSTRACT

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

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

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GENERAL INTRODUCTION

Soil comprises one of the largest reserves of carbon (C) in the biosphere (RIGON; CALONEGO, 2020), storing two thirds of the total terrestrial C (LORENZ; LAL, 2018). However, depending on the management, the soil can act as net sink of C or a net source, emitting greenhouse gases responsible for the global warming. Increasing soil organic C in deeper layers has been considered a strategy to enhance sequestration (LORENZ; LAL, 2018). In addition, recent research indicates that the implementation of conservation agriculture managements such as the no-till system, maintaining soil cover, associated with crop rotation and diversification of plant species (FAO, 2017), increase organic carbon sequestration and mitigation of CO₂ emissions to the atmosphere (RIGON et al., 2021; RIGON; CALONEGO, 2020). A common agricultural practice in Brazil involves growing soybean [*Glycine max* (L.) Merrill] followed by maize (*Zea mays* L.) in the off season. Thus, plants such as tropical perennial grasses are generally cultivated in no-till systems as cover crops, because of its prolonged persistence in the soil surface and their higher C:N ratio (GRASSMANN et al., 2020; ROSOLEM et al., 2017). Thus, to optimize the cropping system the off-season-maize can be grown intercropped with grass species such as Guinea grass (*Megathyrsus maximum* cv. Tanzânia) improving plant biomass production, soil covering and subsoil root exploration (BOSSOLANI et al., 2020; ROCHA et al., 2020).

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

altogether with the removal of cations from the soil during intensive agriculture and natural processes (via C, N and S cycling) may increase soil acidity (HOLLAND et al., 2018; KUNHIKRISHNAN et al., 2016).

Soil acidity is one of the primary factors limiting crop development and productive potential, mainly in tropical areas (BOSSOLANI et al., 2020; HOLLAND et al., 2018; RITCHEY et al., 1980). Low soil pH, cause adverse effects on plants and soil microorganisms, reducing nutrient availability, and restricting plant root development and access to water and nutrient (KUNHIKRISHNAN et al., 2016; RITCHEY et al., 1980). Liming is most common practice to alleviate soil acidity, but due to its low solubility and to the surface application in no-till systems, the reaction may be restricted to the first soil layers (CARMEIS FILHO et al., 2017; FERRARI NETO et al., 2021; RITCHEY et al., 1980). Gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) generally does not affect soil pH, but present higher solubility compared with lime, promoting the movement of sulphate (SO_4^{2-}) through the soil profile carrying cations as Ca^{2+} and reducing Al^{3+} activity, providing a better environment for root growth (CARMEIS FILHO et al., 2017; RITCHEY et al., 1980; VIEIRA FONTOURA et al., 2019). Then, especially in the short-term gypsum can be recommended as a soil conditioner (HOLLAND et al., 2018; RITCHEY et al., 1980; ZOCCA; PENN, 2017). Benefits resulting from the combination of lime and gypsum can include increase subsoil fertility and root growth deep down in soil profile, greater plant biomass production and higher crop yield, increasing C inputs in the soil (CARMEIS FILHO et al., 2017; DA COSTA; CRUSCIOL, 2016; FERRARI NETO et al., 2021; INAGAKI et al., 2017).

In addition, soil pH is tightly associated with shifts in soil microbial community structure (JONES et al., 2019). Low soil pH critically affects bacteria diversity (ZHALNINA et al., 2015), fungi:bacteria ratios (ROUSK; BROOKES; BÅÅTH, 2010), N mineralization (ACIEGO PIETRI; BROOKES, 2008), microbial C use efficiency (CUE) and turnover rates (JONES et al., 2019; MALIK et al., 2018), among others. Furthermore, microbial trade-off to soil acidity stress adaptation and biomass production lead to lower potential for C storage through increased microbial growth efficiency (MALIK et al., 2018). Thus, liming associated with gypsum can have a positive effect on microbial biomass and microbial activity (GARBUIO et al., 2011; INAGAKI et al., 2017; KEMMITT et al., 2006), with subsequent effects on C mineralization and N mineralization throughout the soil profile (GARBUIO et al., 2011; WACHENDORF, 2015). Liming is frequently associated with increase in carbon

dioxide (CO₂) emission during its chemical dissolution by increasing soil biological activity and hence soil respiration (ABALOS et al., 2020; GIBBONS et al., 2014; KUNHIKRISHNAN et al., 2016; WACHENDORF, 2015). Lime and gypsum addition, by decreasing Al³⁺ ions availability, may favour methane (CH₄) oxidizers activity, increasing CH₄ uptake (ABALOS et al., 2020; LINDAU et al., 1994). Repeated applications of lime are likely to increase soil N mineralization, but the occurrence of net mineralization or net immobilization resulting from liming will depend on the C:N ratio and the quality of the organic matter (HOLLAND et al., 2018; WACHENDORF, 2015). In general, liming is associated with decrease in nitrous oxide (N₂O) emission (ABALOS et al., 2020; HOLLAND et al., 2018; KUNHIKRISHNAN et al., 2016), and increases in ammonia (NH₃) volatilization (SMITH et al., 2009; SOMMER; ERSBØLL, 1996) and nitrate (NO₃⁻) leaching (GIBBONS et al., 2014; KEMMITT et al., 2006; ROSOLEM et al., 2017). Therefore, understanding this complex dynamic in addition to the effects N fertilizer addition may be important for the mitigation of climate change, seeking the best management to increase C sequestration and avoid C and N losses in the system.

In the present study we discuss how liming, and the co-addition of gypsum and N fertilizer affect the mechanisms and dynamics of C and N in the system, and its importance for the mitigation of climate change. The positive increments of C and N in the system, in addition to the positive/unexpected effects of treatments on accumulated CO₂-C emissions, pointed out in the first chapter were fundamental for stimulate a search for detailed explanations of how the treatments were affecting soil microbial activity and microbial community profile. The results presented here reveal new perspectives on how the management of lime and gypsum affects the dynamics of C and N inserted in a production system in a short-term through a set of complementary methods, allowing to achieve a holistic understanding of the C and N cycle, and how lime and gypsum may reflect on some of the drivers of climate change.

CHAPTER 1
SOIL ACIDITY AMELIORATION INCREASES SOIL N AND C IN THE SHORT
TERM IN A SYSTEM WITH SOYBEAN FOLLOWED BY MAIZE-GUINEA GRASS
INTERCROPPING

ABSTRACT

Lime application has been associated with N and C losses from agricultural systems through NO_3^- leaching, N_2O emission and CO_2 emissions. However, we hypothesized that liming could increase soil N and C in intensive crop systems where soybean is grown in rotation with maize intercropped with a forage grass, especially with the application of gypsum to ameliorate the subsoil. To evaluate the interaction of lime, gypsum and N fertilizer on soil and N and C balances, an experiment was carried out under no-till, where soybean was grown in rotation with maize intercropped with Guinea grass (*Megathyrsus maximus* cv Tanzânia) as relay crop. Lime and gypsum were applied before soybean planting in October 2016 and October 2017. Two N rates (0, 160 kg ha⁻¹ of N as ammonium sulfate) were applied annually to maize. N and C inputs, outputs and partial N and C balances were determined. N outputs through NO_3^- -N leaching, N_2O -N emission and NH_3 -N volatilization did not exceed 30 kg ha⁻¹ in two years. The N exported in grains consisted in 96% of the N output, and was greater with fertilizer and lime. Soil acidity amelioration and N-fertilization favoured soil C fixation with high plant biomass production (C in plant residue, roots, and grains), while CO_2 -C emission was not affected, resulting in soil C increase. Although the partial N balance was negative, there was an increase in soil N, probably due to biological N fixation, which was not considered in the balance. We concluded that lime and gypsum application to soybean cropped in rotation with maize-guinea grass intercropping benefited the system and the environment in the short-term irrespective of N application. However, in future research, the effect of N fertilization should be considered in the long-term, since this treatment showed great influence over plant biomass and grain production.

Key words: Liming, gypsum, ammonium sulfate, greenhouse gas emission, conservative system

1.1 INTRODUCTION

Conservation agriculture management involves, among other practices, the implementation of systems under no-till, permanent soil cover with crop residues and/or cover crops, plant species diversification and crop rotation (FAO, 2017). Crop residue retention on the soil surface results in several benefits to soil and water conservation. The rotation of cash crops and forage production, and the combination of leguminous and grass species have shown positive effects on N and C inputs in the system (Ferrari Neto et al., 2021; Rigon and Calonego, 2020). Soybean [*Glycine max* (L.) Merrill] grown before maize (*Zea mays* L.) in the off-season is a common cropping system in Brazil. To increase soil covering and/or produce forage to be pastured, an alternative is growing the off-season-maize intercropped with grass species such as Guinea grass (*Megathyrsus maximum* cv. Tanzânia) to improve plant biomass production, and subsoil root exploration (Bossolani et al., 2020; Rocha et al., 2020).

The inclusion of leguminous species in the system is recommended to reduce the dependence on N mineral fertilizer (Lötjönen and Ollikainen, 2017). However, N fertilizer may be critical to sustain or to achieve high-yield levels in maize, especially in crop systems that include intercropping with tropical grasses (Rocha et al., 2020). Biological nitrogen fixation (BNF) by *Rhizobia* sp bacteria associated with legumes and the addition of ammonium-based N fertilizer may increase soil acidity (Ahmad et al., 2020; Nyatsanga and Pierre, 1973), which is one of the primary factors limiting crop development and yield potential, mainly in tropical areas (Holland et al., 2018; Ritchey et al., 1980). Liming is the most common practice to mitigate soil acidity. However, because of the low solubility of lime and due to surface application in no-till systems, the reaction may be restricted to the first soil layers (Ferrari Neto et al., 2021; Ritchey et al., 1980). Although gypsum does not modify soil pH, it decreases Al^{3+} activity throughout the soil profile, providing a better environment for root growth (Ritchey et al., 1980; Vieira Fontoura et al., 2019). Then, especially in the short-term, gypsum can be applied as a complement to ameliorate subsoil fertility (Holland et al., 2018; Ritchey et al., 1980; Zoca and Penn, 2017). Benefits resulting from the combination of lime and gypsum can include greater plant biomass production, denser root system and higher crop yield, and eventually a positive impact on soil C and N (Ferrari Neto et al., 2021; Inagaki et al., 2017).

Soil acidity and liming impact the activities of microorganisms involved in the C and N cycles and their transformations in the soil, therefore affecting greenhouse gas (GHG) fluxes in soils (Kunhikrishnan et al., 2016). The chemical dissolution of lime during soil pH neutralization is considered a net source of atmospheric carbon dioxide (CO₂). The soil biological activity is increased resulting in higher soil respiration (Abalos et al., 2020; Gibbons et al., 2014; Kunhikrishnan et al., 2016; Wachendorf, 2015). Liming has been shown to affect methane (CH₄) oxidation rates and decrease nitrous oxide (N₂O) emission (Abalos et al., 2020; Holland et al., 2018; Kunhikrishnan et al., 2016). In addition, it has been reported that lime application may increase NH₃ volatilization (Smith et al., 2009; Sommer and Ersbøll, 1996) and nitrate loss to the groundwater under inappropriate management (Gibbons et al., 2014; Kemmitt et al., 2006; Rosolem et al., 2017). Despite the increase in biomass and grain yields, nitrogen fertilization can increase CO₂ and N₂O emissions, NO₃⁻ leaching and NH₃ volatilization, and reduce CH₄ oxidation (Grassmann et al., 2020; Kunhikrishnan et al., 2016; Rocha et al., 2020; Wang et al., 2010), affecting both C and N balances in the system.

Although the expected counterbalance by higher biomass production promoted by soil pH neutralization and N availability, intensive agricultural systems can lead to soil N depletion through nutrient removal (Raphael et al., 2016; Rigon and Calonego, 2020; Rocha et al., 2020), impacting negatively soil fertility and resulting in C and soil organic matter loss, eventually resulting in soil degradation (Purwanto and Alam, 2020; Raphael et al., 2016). Thus, we hypothesized that the application of lime in association with gypsum can improve soil C fixation by benefiting soybean BNF, increasing biomass production and N recycling through plant residues. Furthermore, these soil amendments and nitrogen fertilization combined will improve N and C in the system. The objective of this work was to evaluate the effect of the interaction between lime, gypsum and N fertilizer application on N and C inputs and outputs, and on the partial N and C balances in a cropping system with soybean grown in rotation with maize intercropped with Guinea grass as a relay crop.

1.2 MATERIALS AND METHODS

1.2.1 Site characteristics

A field experiment was conducted in Botucatu, São Paulo State, Brazil (770 m a.s.l.; 22° 49'51.6" S; 48° 25'38.6" W). The fine-loamy soil (547 g kg⁻¹ sand, 109 g kg⁻¹ silt, and 343 g kg⁻¹ clay; 0.0-1.0 m soil depth) is a Typic Hapludox (Soil Survey Staff, 2014). The climate of the region is Cwa (mesothermic with dry winters) according to the Köppen classification (Alvares et al., 2013). The mean annual temperature is 23.2°C, with a monthly average maximum of 30 °C and an average minimum of 18 °C. The annual rainfall is 1450 mm (29-year average). Rainfall and temperatures during the experiment (Figure 1) were obtained from a meteorological station close to the site.

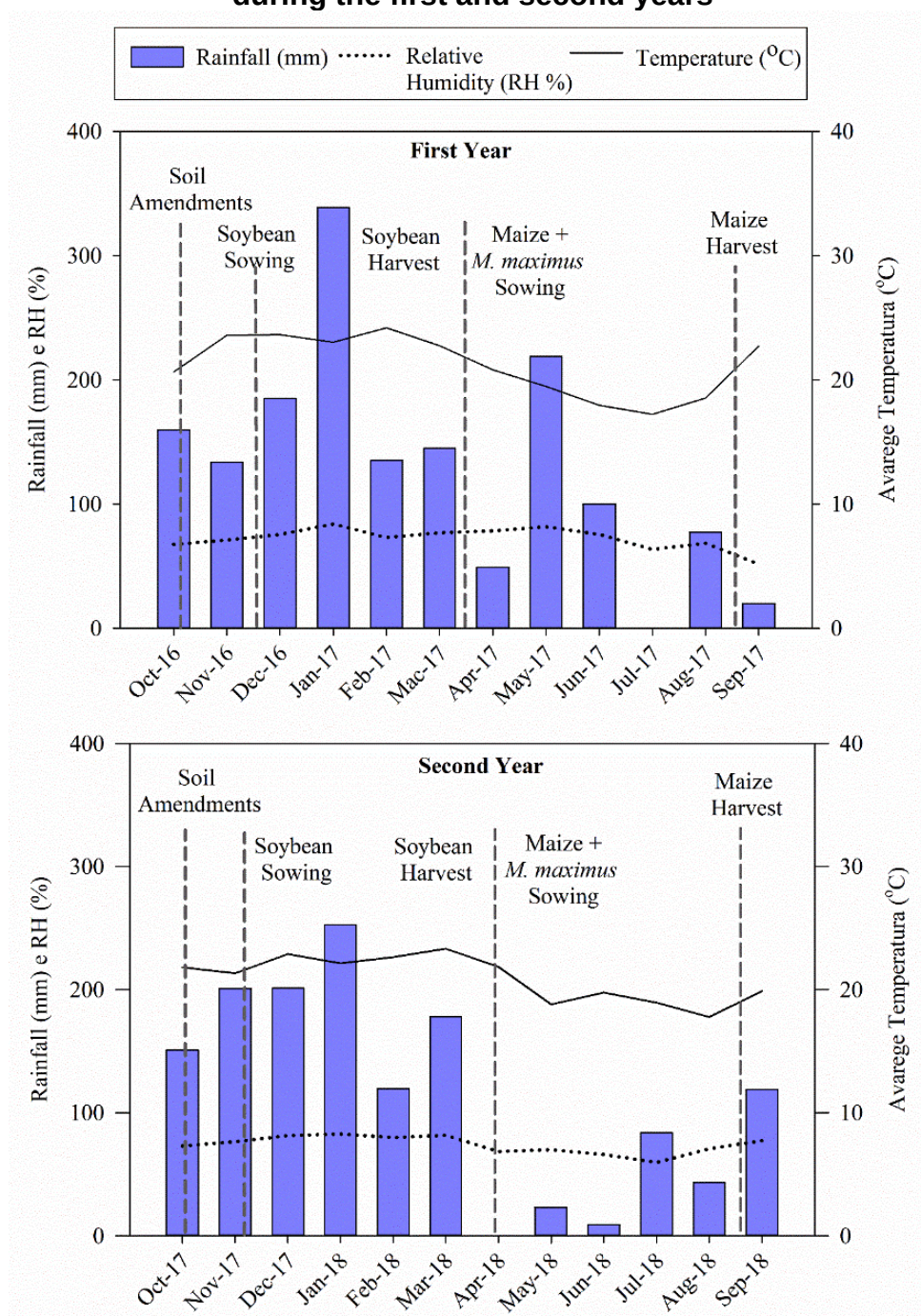
Before the experiment, samples were taken from the 0.0-1.0 m soil depth and selected soil chemical (Table 1) characteristics were determined (Raij et al., 2001). The bulk density in topsoil (0.0-0.10 m) was 1.3 ± 0.1 g cm⁻³.

Table 1 - Selected soil properties before the experiment

Depth	pH	SOM	P	S	K	Ca	Mg	Al	H+Al	EB	CEC	BS
m	CaCl ₂	g dm ⁻³	mg dm ⁻³									%
0.0-0.10	4.38	18	13	5	2.07	12	12	9	52	25.9	78.2	32
0.10-0.20	4.29	14	10	5	1.07	9	8	9	48	18.0	65.6	27
0.20-0.40	4.14	11	6	22	0.46	4	4	12	55	8.2	63.0	13
0.40-0.60	3.96	11	6	21	0.40	3	4	20	90	7.4	97.0	8
0.60-0.80	3.94	12	6	17	0.16	5	4	22	99	8.2	107.2	9
0.80-1.00	3.93	12	5	15	0.25	4	3	23	103	7.4	109.9	8

SOM = Soil organic matter; P = phosphorus available (P_{resin}); K = exchangeable potassium (K⁺); Ca = calcium (Ca²⁺); Mg = magnesium (Mg²⁺); Al = exchangeable aluminium (Al³⁺); H+Al = potential acidity; EB = sum of exchangeable bases; CEC = Cation exchange capacity; BS = base saturation.

Figure 1 - Monthly total rainfall, relative humidity (RH) and average temperature during the first and second years



SOURCE: the author.

1.2.2 Experimental design and treatments

The experiment design was a 3×2 factorial in complete randomized blocks with four replications. The treatments were (i) control without amendments; (ii) 2.92 Mg ha^{-1} of lime and (iii) 2.92 Mg ha^{-1} of lime + 2.06 Mg ha^{-1} of gypsum. These treatments were combined with 0 and 160 kg ha^{-1} of N applied to maize as ammonium sulfate. The lime

rate was calculated to raise soil base saturation (BS) to 60% in the topsoil, and gypsum rate was calculated by multiplying the clay content in the 0.20-0.40 m soil layer by 6.0 (Quaggio and van Raij, 1997). The experiment was conducted under no-till, and lime and gypsum were broadcast on the soil surface before the first soybean crop, and again in October 2017. Soybean was grown in the summer (2016/17 and 2017/18), followed by maize as a relay crop intercropped with Guinea grass (*Megathyrsus maximus* cv Tanzânia). Nitrogen was applied annually to maize.

Prior to the experiment, the area had been cropped with black oats, which was desiccated with 2.0 kg ha⁻¹ (a.i.) of glyphosate. Soybean, cultivar TMG 7062 IPRO (Tropical Breeding & Genetics), was planted in November 2016 and 2017 in rows 0.45 m apart using 250,000 seeds per hectare. Seeds were inoculated 1.0 h before planting with liquid inoculant containing *Bradyrhizobium japonicum* (SEMIA 5079) + *B. diazoefficiens* (SEMIA 5080) using 1.2 ml kg⁻¹. In both seasons, all plots were fertilized with 26 kg ha⁻¹ of P and 50 kg ha⁻¹ of K. Each plot consisted of 10 rows 10-m long and spaced 0.45 m apart. Plots were separated 3.0 m one from another to avoid contamination and to allow for machine traffic.

One day after soybean harvest, in March 2017 and 2018, maize was planted over soybean residues, intercropped with Guinea grass. Maize (Dow AgroScience hybrid 2B 587 RRBTPW) was sown in rows 0.45 m apart, using 70,000 seeds per hectare. Simultaneous to maize sowing, Guinea grass was sown at a density of 9.5 kg ha⁻¹ of pure live seeds at a depth of 0.08 m to avoid early competition with maize. At planting, all plots were fertilized with 26 kg ha⁻¹ of P and 50 kg ha⁻¹ of K and N was side-dressed at 30 kg ha⁻¹ as ammonium sulfate 0.10 m from the sowing line. The balance of N fertilizer, i.e., 130 kg ha⁻¹, was applied to the respective treatments when the plants were at the V4-V5 stage. After maize harvest (August 2017 and August 2018), Guinea grass was left to grow around two months, until the next season. Pest monitoring and management were carried out according to local recommendations for soybean and maize.

1.2.3 Nitrogen input by rainfall

Nitrogen input by rainfall was quantified by assessing the volume of rain and its N concentration, similar to Rocha et al., (2020). There were installed two rain gauges in the experimental area, and, after each rainfall, samples were collected

stored and in glass vials, then refrigerated until analysis. Nitrate (NO_3^- -N) and ammonium (NH_4^+ -N) concentrations were quantified colorimetrically in rain samples (Miranda et al., 2001; Mulvaney, 2018). Total inorganic nitrogen was obtained considering the measured concentrations of mineral forms (NH_4^+ -N and NO_3^- -N).

1.2.4 Nitrate leaching

Nitrate leaching was estimated over the experimental period based on NO_3^- -N concentrations in the soil solution and on the volume of drained water below 0.80 m (Rocha et al., 2020; Rosolem et al., 2018). Soil solution was collected using ceramic cup extractors placed in the center of the plots at a depth of 0.80 m (Reichardt et al., 1977). Vacuum was applied to the cups after rainfall events, and the soil solution was collected after 2-3 days. Samples were stored at 20 °C until analysis. The NO_3^- -N concentration in soil solution was determined by colorimetry (Miranda et al., 2001). The volume of water drained below 0.80 m depth was estimated using a capacitance probe (Diviner 2000®, Sentek Pt Ltd., Australia 2009), measuring soil moisture every 0.10 m up the depth of 1.0 m. Access tubes were installed in the center row of each plot, 0.50 m far from the ceramic cup extractors. Measurements were taken periodically, and the time interval between two consecutive measurements depended on rainfall. The water content exceeding the point of saturation throughout the soil profile was considered drained. Drainage was estimated using IrriMax® (Sentek, Adelaide, Australia). By integrating the amount of water drained at each sampling time, the total drainage below 0.80 m was estimated.

1.2.5 Greenhouse gas emissions and ammonia volatilization

To estimate gas emissions as nitrous oxide (N_2O -N), methane (CH_4 -C) and carbon dioxide (CO_2 -C), 0.30 m diameter static chambers were installed in each plot in the plant inter-rows. Using 20 mL nylon syringes, headspace air samples were collected 0, 10, 20 and 40 min after closing the chambers (Bowden et al., 1991). Samples were always taken from 08:00 to 10:00 am, and soil temperature and moisture were determined at the sampling time. Gas samples were collected at 1, 3, 5, 8, 15, 30 and 60 days after each event (desiccation, soil amendment application, sowing and N fertilizer application), and then monthly up to the next event, when a new

cycle was started (La Scala Jr. et al., 2009). Gas concentrations were determined by gas chromatography (Shimadzu – GC 2014, Greenhouse model, Shimadzu Scientific Instruments, Kyoto, Japan). The results were scaled up to kg ha^{-1} of $\text{N}_2\text{O-N}$, $\text{CH}_4\text{-C}$ and $\text{CO}_2\text{-C}$.

Ammonia ($\text{NH}_3\text{-N}$) volatilization was estimated using open collectors with foam absorbers (Oliveira et al., 2008). Each absorber had an 0.08×0.08 m foam (density of 20 kg m^{-3}) placed on a $0.10 \times 0.10 \times 0.0002$ m acrylic plate. The foam was soaked with 11 mL of a 0.5% phosphoric acid + 4% glycerol solution. To avoid contamination, each absorber was previously prepared, stored in plastic bags and refrigerated until placed in the field. The collectors were placed around 0.20 m over the plant canopy and adjusted as needed. The absorbers were replaced twice weekly, throughout the experiment starting after each event (desiccation, soil amendment application, sowing and N fertilizer application), and then every 15 days until the next event. Collected foams were placed in sealed containers and refrigerated at 4°C or analyzed immediately. For analysis, foams were washed with 100 mL of 0.0005 N sulfuric acid, then an aliquot was taken, and $\text{NH}_3\text{-N}$ concentration was determined by colorimetry (Mulvaney, 2018). The amount of $\text{NH}_3\text{-N}$ captured in the collectors was scaled up to kg ha^{-1} .

1.2.6 Plant nitrogen and carbon content

Plant residues remaining on the soil were collected monthly from two random points per plot using a 0.50×0.50 m wooden frame (Rosolem et al., 2018). The samples were dried to constant weight in a forced air oven at 65°C for 72 h. The dry mass was weighed, and data were extrapolated to Mg ha^{-1} . A subsample was ground in a Wiley mill and sieved through a 1-mm mesh, and the remaining residue was returned to the field. Total N and C plant residue content at the beginning and at the end of each cycle were calculated to assess N and C release (resN and resC).

At soybean flowering, phenological stage R2 (Fehr et al., 1971), and at maize flowering, phenological stage R1 (Ritchie et al., 1993), roots were sampled in three randomly points and combined into one composite sample per plot, at the sowing line and at the inter-row to the depth of 0.60 m, using a 5.5 cm i.d. stainless steel corer. Roots were washed to remove substrate particles and stored in 70% ethanol solution. Total root volume was assessed using an HP Scanjet 4c/T scanner and WinRHIZO

Reg 3.8b-1993-1997 (Regent Instruments, Sainte-Foy, Canada) software. Then, the entire root system was oven-dried at 65°C for 72 h and weighed. Dry samples were ground using pestle and mortar, and total C content in roots was determined by dry combustion with a CHN-2000 analyzer (LECO Corp., St. Joseph, MI, USA).

Soybean and Maize grain yields were determined by harvesting 5.0 m of the three central rows. The yield was estimated at 130 g kg⁻¹ grain moisture content (standard moisture for commercialization of soybean and maize grains in Brazil). A subsample was taken, oven-dried at 65°C for 72 h and ground using a ball-mill to determine total N and C in an automated elemental analyzer (TruSpec™ CHNS, LECO®) (Swift 1996).

1.2.7 Nitrogen and carbon balance

The partial N balance in the system comprised the whole period from the soil amendment application in October 2016 to maize harvest in August 2018. N balance was calculated following equation (1):

$$NBal = (raN + feN + resN) - (NO_3^- - N + N_2O - N + NH_3 - N + grN) \quad \text{Eq. (1)}$$

where: NBal = N balance; raN = N input from rain; feN = N input from fertilizer; resN = N released from plant residue; NO₃⁻-N = N output by nitrate leaching; N₂O-N = N output by nitrous oxide emission; NH₃-N = N output by ammonia volatilization; grN = grain N exported (soybean grain N exported + maize grain N exported).

The partial C balance in the system comprised the whole period from the soil amendment application in October 2016 to maize harvest in August 2018. C balance was calculated following equation (2):

$$CBal = (resC + rootC + grC) - (CO_2 - C) \quad \text{Eq. (2)}$$

where: CBal = C balance; resC = C released from plant residue; rootC = C from roots; grC = C fixed in grains (soybean grain C + maize grain C); CO₂-C = C emission as carbon dioxide.

1.2.8 Soil nitrogen and carbon analysis

Soil samples were taken 30 days after the first application of lime and gypsum in 2016, and in October 2018, using a core sampler at the following depths: 0.0-0.10, 0.10-0.20, 0.20-0.40, 0.40-0.60 and 0.60-0.80 m. Three subsamples were randomly taken and combined into one lab sample per soil depth per plot. Samples were air-dried and homogenized using a 2-mm sieve. Dry samples were ground using a ball mill, and total N and C were determined by dry combustion with a CHN-2000 analyzer (LECO Corp., St. Joseph, MI, USA).

To estimate the soil N accumulation, soil bulk density at each soil depth (0.0-0.80 m depth) and position was assessed using the volumetric ring method (Blake, 2015).

The modification on N stock in soil along the years of the experiment, called Annual Nitrogen Increment in soil was calculated by the following equation (3):

$$ANI \text{ (kg ha}^{-1}\text{)} = ((NF_{STOCK} - NI_{STOCK}) / 2) \times 1000 \quad \text{Eq. (3).}$$

where: NF_{STOCK} = is the N stock from soil collected in 2018; NI_{STOCK} = is the N stock from soil collected in 2016.

The alteration on C stock in soil along the years of experiment, called Annual carbon increment in soil was calculated by the following equation (4):

$$ACI \text{ (kg ha}^{-1}\text{)} = ((CF_{STOCK} - CI_{STOCK}) / 2) \times 1000 \quad \text{Eq. (4).}$$

where: CF_{STOCK} = is the C stock from soil collected in 2018; CI_{STOCK} = is the C stock from soil collected in 2016.

1.2.9 Statistical analysis

All statistical procedures were performed in R (version 3.6.3, The R Foundation for Statistical Computing, Boston, MA, USA) and RStudio (version 1.2.5019, RStudio, Vienna, Austria). Data normality and homoscedastic were analyzed by Shapiro-Wilk and Bartlett tests. Non-normally distributed data were $\log_{10}$ -transformed prior to analysis. Factorial ANOVA based on a complete randomized block design with two factors (correctives, nitrogen rates) was carried out. Fisher's least significant difference

(LSD) test was used to compare means. Statistical significance is reported at the 5% level of significance.

1.3 RESULTS

1.3.1 *Nitrogen balance*

Nitrogen input to the system as wet deposition by rainfall (raN), summed a total of 4.97 kg ha⁻¹ during the experiment (Table 2) with an accumulated rainfall of 2,892 mm (Fig. 1). The N input by fertilizer considered the sum of the two years, with 0 and 320 kg ha⁻¹ of N for the 0 and 160 kg ha⁻¹ of N treatments. Another source of N was the N released from straw decomposition. Treatments with lime applied alone released an average of 5.47% and 12.59% more N from plant residue than control and lime + gypsum, respectively.

The outputs of N from the system, NO₃⁻-N leaching, N₂O-N emission, NH₃-N volatilization and N exported by soybean and maize grains are shown in Table 2. The NO₃⁻-N leaching was higher for lime application with N fertilizer reaching 1.39 kg ha⁻¹ in two years. The highest N₂O-N emission was observed in the N fertilized treatment without lime or gypsum, and there was no difference between limed treatments, disregard of N fertilizer application. Accumulated N₂O-N emissions did not exceed 0.75 kg ha⁻¹. NH₃-N volatilization was higher for treatments with N fertilizer and with lime. The total N export in soybean and maize grains was greater with N fertilizer, irrespective of amendment application. Lime applied alone showed greater total N export in soybean and maize grains than control and lime + gypsum, irrespective of N fertilization.

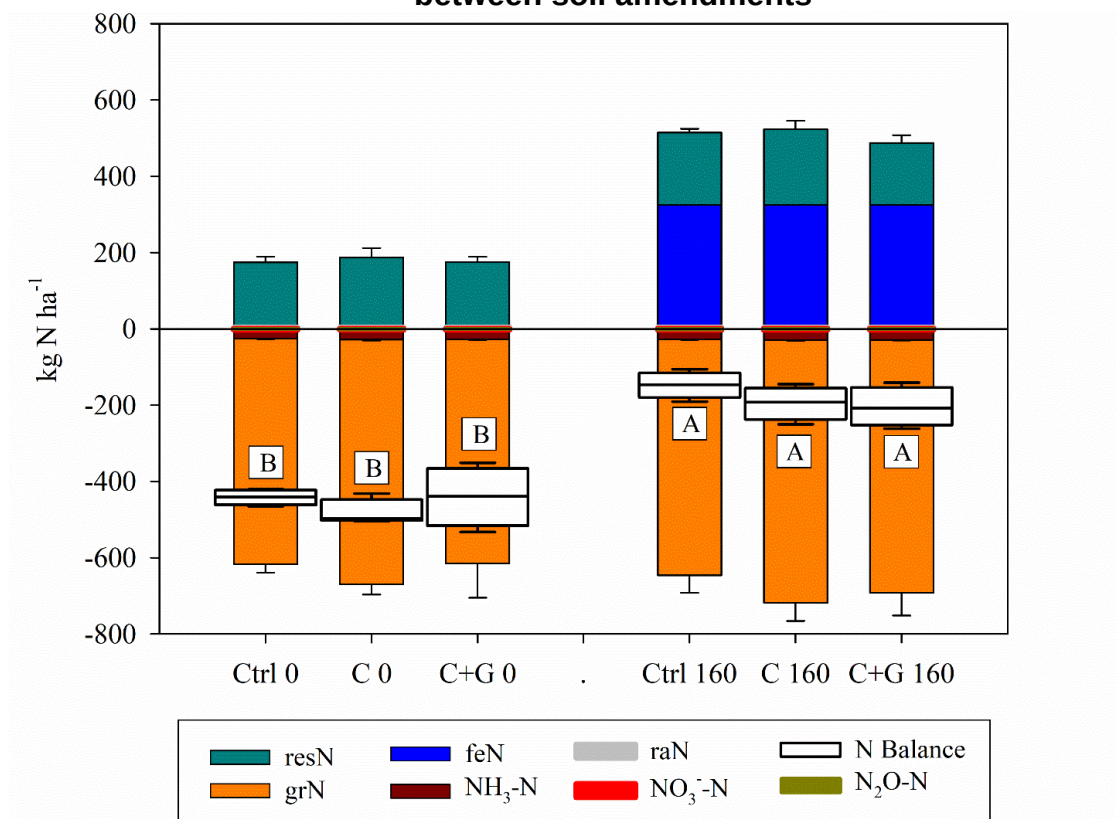
Table 2 - Average N inputs from rain (raN), N from fertilizer (feN) and N released from plant residue (resN) and NO_3^- -N leaching (NO_3^- -N), N_2O -N emission (N_2O -N), NH_3 -N volatilization (NH_3 -N) and grain N exported (grN) accumulated in two years. (n = 4)

N Balance – Inputs					
Corrective	Fertilizer	raN	feN	resN	
kg of N ha ⁻¹		kg N ha ⁻¹			
Control	0	4.97	0	169.92	ab
	160	4.97	320	189.80	ab
Lime	0	4.97	0	182.05	a
	160	4.97	320	198.48	a
Lime +Gypsum	0	4.97	0	170.11	b
	160	4.97	320	162.53	b
MDD					
Corrective (C)	-	-	-	17.18	*
Fertilizer (N)	-	-	-	14.03	ns
C×N	-	-	-	24.30	ns
N Balance – Outputs					
Corrective	Fertilizer	NO_3^- -N	N_2O -N	NH_3 -N	grN
kg of N ha ⁻¹		kg N ha ⁻¹			
Control	0	1.05	0.62 B	24.04 b B	591.21 b B
	160	1.23 ab	0.75 a A	24.89 b A	619.49 b A
Lime	0	0.98 B	0.69	26.24 a B	641.62 a B
	160	1.39 a A	0.67 b	27.40 a A	689.18 a A
Lime +Gypsum	0	1.16	0.69	25.18 a B	557.42 b B
	160	1.04 b	0.65 b	27.32 a A	652.11 b A
MDD					
Corrective (C)	0.21 ns	0.04 ns	1.66 *	45.28 *	
Fertilizer (N)	0.17 ns	0.03 *	1.35 *	36.97 *	
C×N	0.29 *	0.06 *	2.34 ns	64.03 ns	

Different letters indicate differences according to LSD's HSD post-hoc ($p < 0.05$). Uppercase letters indicate differences between nitrogen levels, while lowercase letters indicate difference between soil amendments. MDD = minimal detectable difference.

The partial N balance in the system was negative and more negative for treatments without N (Figure 2). On average, N balance for treatments without N was -455.06 kg ha⁻¹ accumulated in two years, against -182.42 kg ha⁻¹ for treatments that received 160 kg ha⁻¹ of N per year.

Figure 2 - Partial nitrogen balance in cropping systems with soil amendments and two N rates (0 and 160 kg ha⁻¹ of N) accumulated in two years. Average (*n* = 4). Ctrl = Control; L = Lime; L+G = Lime + Gypsum; 0 = 0 kg ha⁻¹ of N fertilizer; 160 = 160 kg ha⁻¹ of N fertilizer. Different letters indicate differences according to LSD's HSD post-hoc (*p* < 0.05) for N Balance. Uppercase letters indicate differences between nitrogen levels, while lowercase letters indicate difference between soil amendments



SOURCE: the author.

1.3.2 Carbon balance

The sum of C released from the plant residue decomposition during the experiment was 11.84% greater for treatments that received N fertilizer, irrespective of lime or gypsum application (Table 3). Lime applied alone, without N fertilizer, showed higher C input by root deposition with 1116.32 kg ha⁻¹ of C. However, there was no difference in C input by roots between control, lime and lime + gypsum when N fertilizer was applied. The amount of C accumulated in grains of soybean and maize was 10.4% higher in presence of N fertilizer, irrespective of soil amendments. Comparing soil amendments, limed treatments had 11.9 % more C accumulated in grains than the control, irrespective of N fertilizer.

Table 3 - Average C inputs as C released from plant residue (resC), from roots (rootC) and carbon fixed in grains of soybean and maize and C output by methane emission (CH₄-C) and by carbon dioxide emission (CO₂-C) accumulated in two years. (n = 4)

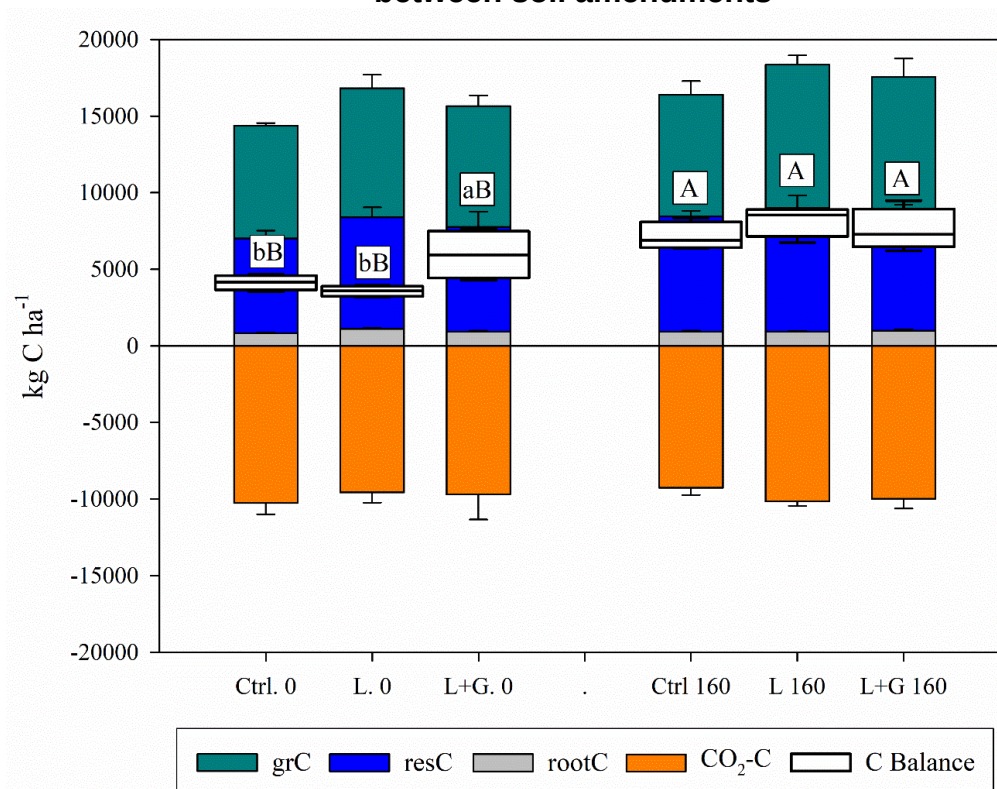
C Balance – Inputs							
Corrective	Fertilizer	resC	rootC	grC			
kg of N ha ⁻¹		kg C ha ⁻¹					
Control	0	6190.33 B	818.85 c B	7377,79	b B		
	160	7520.99 A	927.20 A	7950,99	b A		
Lime	0	7271.24 B	1116.32 a A	8435,09	a B		
	160	8052.41 A	933.16 B	9367,44	a A		
Lime + Gypsum	0	6838.51 B	937.38 b	7875,24	ab B		
	160	7453.39 A	993.02	9106,96	ab A		
<i>MDD</i>							
Corrective (C)		723.86 ns	70.20 *	831,64	*		
Fertilizer (N)		591.05 *	37.32 ns	679,03	*		
C×N		1023.73 ns	99.28 *	1176,12	ns		
C Balance – Outputs							
Corrective	Fertilizer	CH ₄ -C*	CO ₂ -C				
kg of N ha ⁻¹		kg C ha ⁻¹					
Control	0	-0.59 a A	10258.00				
	160	-0.84 b B	9264.70				
Lime	0	-0.98 b B	9578.30				
	160	-0.68 a A	10159.00				
Lime + Gypsum	0	-0.99 b	9695.20				
	160	-0.90 b	9988.00				
<i>MDD</i>							
Corrective (C)		-0.07 *	865.23 ns				
Fertilizer (N)		-0.06 ns	706.46 ns				
C×N		-0.10 *	1223.60 ns				

Different letters indicate differences according to LSD's HSD post-hoc ($p < 0.05$). Uppercase letters indicate differences between nitrogen levels, while lowercase letters indicate difference between soil amendments. MDD = minimal detectable difference.

The CH₄-C flux was negative (ie, CH₄ oxidation) in all treatments, with greater uptake for lime + gypsum, with an average of 0.95 kg ha⁻¹ of C, irrespective of N fertilizer. However, in the absence of N, the control showed lower uptake of CH₄-C, which was higher with lime alone. There was no difference between treatments for CO₂-C emission.

The partial C balance in the system was positive, and greater in treatments with N, with no response to lime and gypsum. Nevertheless, for treatments without N, the C balance was more positive for lime + gypsum, with an 8,194 kg ha⁻¹ increment of C in the system (Figure 3).

Figure 3 - Partial carbon balance in cropping systems with soil amendments and two N rates (0 and 160 kg ha⁻¹ of N) accumulated in two years. Average ($n = 4$). Ctrl = Control; L = Lime; L+G = Lime + Gypsum; 0 = 0 kg ha⁻¹ of N fertilizer; 160 = 160 kg ha⁻¹ of N fertilizer. Different letters indicate differences according to LSD's HSD post-hoc ($p < 0.05$) for C Balance. Uppercase letters indicate differences between nitrogen levels, while lowercase letters indicate difference between soil amendments



SOURCE: the author.

1.3.3 Nitrogen and carbon increment in the system solo

The Annual Nitrogen Increment in soil (ANI) was around 2 Mg ha⁻¹ of N in all treatments (Table 4). Plant residue accumulated from 205.15 to 220.07 kg ha⁻¹ of N during the experiment but was not different between treatments. However, the N remaining in plant residues covering the soil (resN-F) at the end of the experiment was higher in the control without N and for lime + gypsum with N.

There was an increase in the C accumulated in soil for all treatments, with a greater increment for treatments with lime and lime + gypsum. Treatments with N had a greater amount of C accumulated in plant residue during the experiment, irrespective of soil amendments. However, final C content in plant residue was greater for lime + gypsum compared with control and lime applied alone and was not affected by N fertilizer application.

Table 4 - Average of Annual Nitrogen Increment in soil (ANI), N accumulated in plant residue (resN-A) and final N content in plant residue (resN-F), Annual Carbon Increment in soil (ACI), C accumulated in plant residue and final C content in plant residue (resC-F) in two years. Average ($n = 4$)

Corrective	Fertilizer	ANI	resN-A	resN-F
	kg of N ha ⁻¹		kg N ha ⁻¹	
Control	0	2053.54 b	211.28	41.36 a A
	160	2108.17 b	205.15	20.74 b B
Lime	0	2328.77 ab	212.07	24.15 b
	160	2165.41 ab	213.99	20.34 b
Lime + Gypsum	0	2328.60 a	201.59	25.19 b B
	160	2205.23 a	220.07	57.53 a A
<i>MDD</i>				
Corrective (C)		172.08 *	22.56 ns	5.41 *
Fertilizer (N)		140.50 ns	18.42 ns	4.42 ns
C×N		243.36 ns	31.91 ns	7.66 *
Corrective	Fertilizer	ACI	resC-I	resC-F
	kg of N ha ⁻¹		kg C ha ⁻¹	
Control	0	1574.17 b	11217.80 B	5027.47 ab
	160	2286.58 b	11974.86 A	4453.87 ab
Lime	0	3670.81 a	11265.62 B	3994.38 b
	160	3686.07 a	12530.63 A	4478.22 b
Lime + Gypsum	0	3085.07 a	11831.02 B	4992.50 a
	160	3221.41 a	13057.12 A	5603.73 a
<i>MDD</i>				
Corrective (C)		841.40 *	723.89 ns	665.49 *
Fertilizer (N)		687.00 ns	591.05 *	543.37 ns
C×N		1189.92 ns	1359.12 ns	941.14 ns

Different letters indicate differences according to LSD's HSD post-hoc ($p < 0.05$). Uppercase letters indicate differences between nitrogen levels, while lowercase letters indicate difference between soil amendments. MDD = minimal detectable difference.

1.4 DISCUSSION

In our study, the main N input in the system was the fertilizer, and the N released by plant residue was the main source of N for treatments without N fertilizer. Plant residue decomposition and N release from plant residues depend on many factors, like C:N ratio, cellulose, and lignin content (Wachendorf, 2015). In general, low C:N ratios or N fertilizer application can accelerate N release from plant residues, however, this is still debatable (Rosolem et al., 2017). Treatments that received lime alone released a greater amount of N, probably due to the benefits of lime on soybean grown and consequently on the amount of plant residue (Table 2). The N input by wet deposition was small, accumulating around 5.0 kg ha^{-1} of N in two years, while the average observed in another study in the same region was around $4.3 \text{ kg ha}^{-1} \text{ year}^{-1}$ (Rocha et al., 2020). Despite the regular amount of rainfall during the experiment (Figure 1), N concentration in the water was extremely low.

The soil N mineralization can be favored by the increase in pH with lime (Holland et al., 2018), and positive correlations between liming and soil N nitrification have been reported (Curtin et al., 1998; Kemmitt et al., 2006). We observed higher NO_3^- -N leaching for treatments with lime associated with N fertilizer. Under excessive rainfall, the impact of lime on soil nitrification may result in increased NO_3^- leaching (Holland et al., 2018). In a recent study with crop rotation and no N fertilizer addition, Rosolem et al. (2018) found NO_3^- -N leaching above 17 kg ha^{-1} , and the higher losses of N for systems were observed with legume plants. In our experiment, soybean was cropped in rotation with Guinea grass and maize, both known to have deep roots and high demand for N, which resulted in low NO_3^- -N leaching (Rocha et al., 2020; Rosolem et al., 2017), which did not exceed 1.5 kg ha^{-1} in two years (Table 2), similar to the amount observed by Rocha et al. (2020).

N fertilizer is known to increase N_2O emission (Kunhikrishnan et al., 2016). Grassmann et al. (2020) observed increased, yet low fluxes N_2O following N fertilizer application, and the accumulated N_2O emission was also higher under N fertilization. However, increasing soil pH through liming may offer mechanisms to mitigate N_2O emissions (Kunhikrishnan et al., 2016), as can be observed in Table 2. When fertilizer was applied, limed treatments emitted less N_2O -N. Studying different lime rates in Danish soils, Abalos et al. (2020) observed decreased N_2O emission, since denitrification was lower for limed treatments. N emissions from the soil, as N_2O and

N₂, may happen by denitrification, especially under anaerobic conditions (Holland et al., 2018), and are correlated with waterlogged conditions, which is low in well-drained tropical soils (Zaman et al., 2007). Mulvaney et al. (1997) reported that ammonium-based fertilizers with soil acidifying effects produce a higher N₂O to N₂ ratio compared with alkalinity-promoting fertilizers. As N₂ produced in denitrification is frequently correlated with N₂O-N emissions we assume that nitrification must have been low based on our measured N₂O fluxes (Rocha et al., 2020). Another key gaseous process is ammonium volatilization (Holland et al., 2018). Generally, NH₃ volatilization occurs under soil alkalinity conditions, and liming is known to raise NH₃-N emissions (Holland et al., 2018; Smith et al., 2009; Sommer and Ersbøll, 1996). The use of ammonium sulfate is not conducive to ammonia volatilization (Chambers and Dampney, 2009). However, different from what Grassmann et al. (2020) observed, in the present study treatments that received N as ammonium sulfate also had increased NH₃-N emissions. The NH₃-N volatilization derived from senescing plants after grass desiccation is considered an important N loss from the system (Damin et al., 2008). There are reports that this loss is proportional to the amount of N accumulated in plant tissue, and the range estimated from 11 to 17 kg ha⁻¹ (Castoldi et al., 2014), was similar to what we observed, from 11 to 15 kg ha⁻¹ per year, summing from 24 to 26 kg ha⁻¹ of NH₃-N volatilization in two years (Table 2).

The partial N balance was negative for all treatments, and since N fertilizer was the main input in the system, treatments with N were less negative than the control (Figure 2). The outputs as NO₃⁻-N, N₂O-N and NH₃-N together accumulated 10% of total output in the system, while grain N exportation summed around 90% (Table 2). The benefits of liming on nutrient availability for crops, and its impact on grain and biomass yield of arable and grassland crops are well known (Ferrari Neto et al., 2021). Growing maize and ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins.] intercropped, Bossolani et al. (2020) observed a higher N leaf content for both crops and greater maize grain yield with liming and lime + gypsum. In our experiment, although the grass species was different, a greater amount of N was exported in treatments with lime and those receiving N as a consequence of the higher yield (Table 2).

Furthermore, lime influenced positively the C balance (Figure 3) by increasing C accumulation in grains (Table 3). The application of 160 kg ha⁻¹ of N also showed a positive effect on C accumulated in grains, which ranged from 7,378 kg ha⁻¹ of C in the

control to 9,367 kg ha⁻¹. Under higher soil nutrient availability, the plant shifts resource allocation from belowground to aboveground compartments causing a reduction in total root mass, while plants growing in poor environments tend to develop larger root systems to maximize nutrient absorption (Heyburn et al., 2017). This could be observed especially in the control treatments, which showed similar C content with amended soil treatments when N applied, but lower grain C accumulation (Table 3). Root-derived plant residues and rhizodeposition play a significant role in subsoils (Inagaki et al., 2017; Rosolem et al., 2017). In addition, while N released by plant residues have responded to soil amendment application (Table 2), C released by plant residues were greater for treatments with N (Table 3). The C accumulation in plant residue was also greater in treatments higher in N (Table 4). Studying the effect of different crop rotations on C balance, and C and N stock in soil without N fertilization, Rigon and Calonego (2020) observed greater inputs of C and N by plant residues, and higher C and N stock in soil when Sunn Hemp (a legume) was introduced in a soybean/Triticale rotation.

Methane assimilation is common in upland dry soils (Kunhikrishnan et al., 2016), and was observed in all the treatments (Table 3). While liming is related to increases in CH₄-C uptake (Abalos et al., 2020), ammonium-based fertilizer is related to decreased C-CH₄ uptake (Grassmann et al., 2020). Our experiment has shown increased CH₄-C uptake with liming, even on N fertilized treatments. Because of its high sulfate content, gypsum application is frequently suggested as a method to mitigate CH₄-C emission (Lindau et al., 1994), which was not observed in our experiment. Despite the effect of lime on CH₄-C oxidation, and its importance for GHGs emissions (Holland et al., 2018; Kunhikrishnan et al., 2016), the proportional amount of CH₄-C oxidation accumulated on the system, was much lower compared with the CO₂-C accumulated emissions (CH₄-C < -0.99 kg C ha⁻¹ and CO₂-C ~ 9823.87 kg C ha⁻¹). Therefore, we choose not to take it into account in the C Balance calculation.

The pH increase by liming is considered a net source of atmospheric CO₂, not only because of the chemical dissolution of lime but also due to the increased soil biological activity and respiration (Holland et al., 2018; Kunhikrishnan et al., 2016; Wachendorf, 2015). In a field experiment in UK, Gibbons et al. (2014) found that despite the benefit of liming in reducing NO₃⁻-N leaching and N₂O-N emission, the net climate change impact was negative because of the CO₂ emissions as soil acidity was neutralized. Studies suggested that high pH in soil and ammonium-based fertilization

significantly enhance CO₂ emissions (Kunhikrishnan et al., 2016; Wang et al., 2010). However, in the present study, the accumulated CO₂-C emission was not affected by liming or by ammonium-based fertilization (Table 3), because it was compensated by greater plant growth and yield.

The C balance was positive for all treatments, being greater in treatments with 160 kg ha⁻¹ of N (Figure 3). Rigon and Calonego (2020) observed a better C net budget and C balance for a triticale – soybean – Sunn Hemp – crop rotation. The authors suggested that the use of legumes as cover crops, and the consequent increase of N in soil could be considered a key factor in C sequestration. In our experiment, the annual soil carbon increment responded to soil amendments (Table 4) irrespective of N fertilizer improvement on C biomass (Table 3, 4) and its positive effects on C balance (Figure 3). Inagaki et al. (2017) and Ferrari Neto et al. (2021) also reported increments in soil C with lime and lime + gypsum application.

Since the C/N ratio in the soil is quasi-constant (Raphael et al., 2016; Rocha et al., 2020), despite a negative N balance (Figure 2), an increase of 2 Mg was obtained in the soil N stock, with a greater increment for treatments with lime and lime + gypsum (Table 4). The main output in the N balance was N grain exportation. In a study with maize following guinea grass as a cover crop, Rocha et al. (2020) obtained a balance of -94.0 and -20.0 kg ha⁻¹ of N, with 0 and 140 kg ha⁻¹ N applied to maize, respectively. The average N balance we observed in a year was -230.0 and -91.0 kg ha⁻¹ of N, with 0 and 160 kg ha⁻¹ N applied to maize intercropped with guinea grass, respectively. It is worth noticing that our system used two cash crops. In addition, soybean has a high quantity of N, mainly as grain protein (50–60 g N kg⁻¹), and most of it comes from biological nitrogen fixation (Hungria and Mendes, 2015), against 10% crude protein on maize grains (estimated at 16 g N kg⁻¹) (Rocha et al., 2019), resulting in greater N exportation by soybean. Symbioses can meet the N demand of high-yielding soybean (estimated in 300 kg ha⁻¹) and release from 20 to 30 kg ha⁻¹ of N residues into the soil (Holland et al., 2018; Hungria and Mendes, 2015). This explains why, although the N balance was less negative for treatments high in N, this was not reflected in the N released or accumulated by plant residues, or on annual nitrogen increment in soil.

1.5 CONCLUSION

Lime and gypsum application promoted, together with N fertilizer, higher N exportation and C fixation by grains. However, N and C released and accumulated on plant residues only responded to fertilizer application. Grain N exportation composed the main output on N balance, which was negative. CO₂-C accumulated emissions were equal for all treatments. As a consequence of N fertilizer on C accumulated in plant residues and grains, C balance was greater for treatments with N. Although the negative partial N balance, there is an increment in soil N due to biological nitrogen fixation. C increment in soil was also positive and responded to soil amendment application, independent of N fertilizer. In general, the system benefits from soil acidity correction and the succession of leguminous and maize-guinea grass intercropping, with greater N and C accumulation in the soil and less GHG emitted to the atmosphere.

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CHAPTER 2

TOPSOIL AND SUBSOIL C AND N TURNOVER ARE AFFECTED BY SUPERFICIAL LIME AND GYPSUM APPLICATION IN THE SHORT TERM

ABSTRACT

Subsoil acidity alleviation increases carbon (C) accumulation in deeper layers by stimulating root growth and C and nitrogen (N) inputs at depth. However, the effects of lime and gypsum combined with N fertilization on soil CO₂ emissions in tropical soils remains controversial. We evaluated the effects of superficial lime and gypsum application and N-fertilizer on C and N dynamics and microbial C use efficiency (CUE) in samples taken from the topsoil (0-10 cm) and subsoil (40-60 cm) of a no-till field experiment. We evaluated rates of soil C and N mineralization and microbial CUE. ¹⁴C-glucose was used as a natural abundance tracer to assess CUE, while ¹⁴C-arginine was used to assess net C and N mineralization. In the short-term (2 y), liming increased topsoil pH but had no impact on subsoil acidity. Larger amounts of organic C and total N, and increased C-to-N ratios and microbial biomass C and N were found in the topsoil, however, cumulative rates of ¹⁴CO₂ emission were similar in the two soil layers. The addition of lime and gypsum, in isolation or in combination, had no effect on microbial CUE when processing ¹⁴C-glucose. Liming resulted in higher potential N mineralization, and when combined with gypsum and N resulted in higher nitrification in the subsoil. Despite the increased CO₂ emission in the short-term from lime and gypsum application, there was no difference in CUE irrespective of N fertilizer management or soil layer. Furthermore, differences in the mineralization of ¹⁴C-arginine appeared unrelated to soil pH. We conclude that while lime and gypsum may ameliorate soil acidity, it has minimal impact on the cycling of C through the microbial biomass, particularly in the subsoil.

Key words: soil acidity, ammonium sulphate, ¹⁴C-glucose, ¹⁴C-arginine, carbon use efficiency

2.1 INTRODUCTION

Soil is an important C sink, storing two thirds of the C in the terrestrial biosphere (Lorenz and Lal, 2018), however, it can also be responsible for emitting greenhouse gases, such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), known to affect global warming (Pachauri et al., 2014). Soil respiration is largely controlled by the microbial communities activity, which are typically limited by the availability of labile C and N forms (de Sosa et al., 2018; Glanville et al., 2016). Recent studies suggest that the mechanisms regulating C and N dynamics differ through the soil profile and, albeit controversial, that the factors controlling C storage in topsoil and subsoil may be different (de Sosa et al., 2018; Jones et al., 2018b). The main processes by which labile C and organic labile N enters the soil are via root exudation as well as by root and mycorrhizal hyphal turnover (i.e., mineralization of soil organic matter; SOM) (Glanville et al., 2016; Jones et al., 2018b; Struecker et al., 2016). In a range of studies, root abundance, microbial biomass, and basal respiration have all been found to decrease with soil depth (Inagaki et al., 2017; Jones et al., 2018b). This can be further impaired by excess acidity and toxic levels of Al³⁺ in the subsoil which inhibit root growth (Aye et al., 2018; de Sosa et al., 2018).

Liming is highly effective at alleviating soil acidity, but due to its chemical dissolution, lime is considered a net source of CO₂ to the atmosphere, beyond that, the increase in soil pH by lime may increase soil biological activity and hence SOM turnover and soil respiration (Pachauri et al., 2014; Ritchey et al., 1980; Wachendorf, 2015). In addition, due to the poor solubility of most forms of lime, its dissolution and pH amelioration effect may be spatially restricted to the topsoil, especially in the short-term (Ritchey et al., 1980; Vieira Fontoura et al., 2019). It has been reported that the effect of liming on the soil microbial biomass is frequently restricted to the top 5 cm in a tropical soil under no-till (Ferrari Neto et al., 2021). Although gypsum generally does not affect soil pH, its higher solubility compared with lime promotes the movement of SO₄²⁻ through the soil profile carrying Ca²⁺ which helps alleviate Al³⁺ activity, promoting subsoil fertility and rooting (Ritchey et al., 1980; Vieira Fontoura et al., 2019). The low solubility and movement of lime in the soil profile has led to the recommendation of gypsum (CaSO₄·H₂O) as a soil conditioner alongside lime (Ritchey et al., 1980), especially for no-till systems (de Vargas et al., 2019; Vieira Fontoura et al., 2019).

Therefore, a combination of lime and gypsum may promote subsoil fertility and increase root growth deeper into the soil profile (Pivetta et al., 2019; Vieira Fontoura et al., 2019), increasing C inputs into the subsoil (Inagaki et al., 2017; Jones et al., 2018b). Thus, liming associated with gypsum is expected to have a positive effect on the size of the microbial biomass and its metabolic activity (Garbuio et al., 2011; Inagaki et al., 2017; Kemmitt et al., 2006), with subsequent effects on C mineralization and N mineralization throughout the soil profile (Garbuio et al., 2011; Wachendorf, 2015).

Some authors have suggested that subsoil microbial communities are more C efficient due to a permanent limitation of available substrate and/or N limitation (de Sosa et al., 2018; Jones et al., 2018b; Struecker et al., 2016). Thus, the adoption of practices to increase soil C reserves deeper in the soil profile where O₂ concentration are lower, may represent an important strategy for long-term C accumulation, helping to mitigate climate change (Lorenz and Lal, 2018). Considering that lime and gypsum combined with N-fertilizer application may improve subsoil activity and remove N limitation for the microbial community, we hypothesized that surface application of lime and gypsum would alleviate soil acid stress (from Al³⁺/H⁺) and promote greater microbial C use-efficiency (CUE) in both the topsoil and subsoil. Further we hypothesized that the co-addition of lime and gypsum would enhance microbial subsoil responses in comparison to lime alone.

2.2 MATERIAL AND METHODS

2.2.1 *Site characteristics and treatments*

Soil was collected from a no-till field experiment in Botucatu, State of São Paulo, Brazil (770 m a.s.l.; 22° 49'51.6" S; 48° 25'38.6" W). The topsoil (0-10 cm) had a fine-loamy texture and contained 547 g kg⁻¹ sand, 109 g kg⁻¹ silt, and 343 g kg⁻¹ clay. The soil is classified as a Typic Hapludox (Soil Survey Staff, 2014). The mean annual temperature is 23.2°C, and the mean annual precipitation is 1400 mm y⁻¹ (29-yr average). The bulk density in the topsoil (0-10 cm) is 1.3 ± 0.1 g cm⁻³, while in the subsoil (40-60 cm) it is 1.2 ± 0.1 g cm⁻³.

The field experiment consisted of a 3 × 2 factorial design in complete randomized blocks with four replications. The “soil corrective” factor comprised three

levels, namely: (i) control (without application of correctives); (ii) 1.45 Mg ha⁻¹ yr⁻¹ of lime; and (iii) 1.45 Mg ha⁻¹ yr⁻¹ of lime + 1.05 Mg ha⁻¹ yr⁻¹ of gypsum. The “fertilizer” factor was composed of (i) 0 kg ha⁻¹ yr⁻¹ of N (here defined as without N) and (ii) 160 kg ha⁻¹ yr⁻¹ of N (here considered as residual N). The trial had been cropped with soybean [*Glycine max* (L.) Merrill] in the wet summer (November-March), immediately followed by maize (*Zea mays* L.) intercropped with Guinea grass (*Megathyrsus maximus* Jacq. cv Tanzânia) in the dry off-season. After the maize harvest (August), Guinea grass remained in the field as a cover crop, with subsequent desiccation (October) before the next soybean planting. Lime and gypsum were applied annually (October) before the soybean crop at a rate of 1.45 and 1.05 Mg ha⁻¹ yr⁻¹, respectively. The lime rate was calculated to raise soil base saturation (BS) to 60% in the topsoil (Quaggio and van Raij, 1997). The gypsum rate was calculated by multiplying the clay content in the 20-40 cm subsoil layer by 6.0 (Quaggio and van Raij, 1997). In addition, N fertilizer was applied annually to maize + Guinea grass as ammonium sulfate at 0 and 160 kg ha⁻¹ yr⁻¹ (30 kg ha⁻¹ at sowing and 130 kg ha⁻¹ top-dressed to the respective treatments when maize plants were at the V4-V5 stage).

2.2.2 Soil sampling and sample preparation

In the second dry-season, with the presence of Guinea grass (August 2018), three samples were randomly taken from each field plot from depths of 0-10 cm (topsoil) and 40-60 cm (subsoil) using a 5.5 cm i.d. stainless steel corer and combined into one composite sample per soil layer per plot. The soil was passed through a 5 mm mesh to remove roots and macrofauna. This mesh size was used to minimize disturbance to the microbial community (Jones and Willet, 2006). Prior to experimentation the soil water content was measured gravimetrically (24 h at 105 °C), and the water holding capacity (WHC) of each soil layer was determined. Samples were pre-incubated at 60% of WHC and kept stored at 4 ± 1 °C, and rewetted when necessary. Soil carbonate (CO₃²⁻) content was measured with a FOGL bench-top soil calcimeter (BD Inventions P.C., Thessaloniki, Greece) to confirm that no lime-derived CO₃²⁻ remained in the soil. Soluble C and N were extracted from moist soil (5 g) with 25 ml of 0.5 M K₂SO₄ (200 rev min⁻¹, 30 min), followed by storage of the extracts at 4 °C (Jones and Willet, 2006).

2.2.3 Soil analysis

Prior to incubation assays, on moist soil, pH and electrical conductivity (EC) were measured in a 1:5 (w/v) soil-to-distilled water suspensions. Microbial biomass-C (MBC) and -N (MBN) were determined by the chloroform-fumigation extraction procedure of Vance et al. (1987). In the fumigated soil samples were determined MBC and MBN, while dissolved organic C (DOC) and total dissolved N (TDN) were determined in the unfumigated samples. Fumigated and unfumigated samples were extracted in a 0.5 M K₂SO₄ solution (1:5 soil-to-K₂SO₄ solution; w/v), and determined using a Multi N/C 3100[®] analyzer (Analytik Jena, Jena, Germany). MBC and MBN were calculated using a extraction efficiency value (k_{EC} , k_{EN}) of 0.45 (Kemmitt et al., 2006). Ammonium and nitrate in the K₂SO₄ extracts were determined colorimetrically following the salicylic acid procedure of Mulvaney (2018) and vanadate method of Miranda et al. (2001), respectively. Dissolved organic N (DON) was calculated as the difference between TDN and dissolved inorganic N (NH₄⁺-N + NO₃⁻-N). Sub-samples were air-dried, ground using a ball-mill, and total C and N (TN) determined using a CHN-2000 analyzer (Leco Corp., St. Joseph, MI, USA). Since no carbonate-C was found in soil, we assumed that all the C is present in organic forms and therefore designated this major pool as organic C (OC).

2.2.4 Microbial substrate carbon use efficiency

To determine the Mic_{CUE} , a trace amount of ¹⁴C-labelled glucose (10 nM) was added to the soil. The addition of a low rate of C was chosen to replicate substrate concentrations and turnover rates naturally found in the soil (Glanville et al., 2016; Jones et al., 2018b). Similar to Glanville et al. (2016), we assumed that the concentration of substrate added (< 10 nM) did not significantly alter the intrinsic glucose concentration in the soil and was insufficient to induce microbial growth above that occurring naturally, keeping the C flow in the soil at quasi-steady state. Glucose was chosen as a model compound as it represents the dominant C substrate entering soil, either in a monomeric or polymeric form.

For each sample, 5 g of soil was placed into sterile 50 cm³ polypropylene containers. Subsequently, 0.25 ml of ¹⁴C-labeled D-glucose (Sigma-Aldrich Ltd., Poole, UK) at 10 nM and 3.5 MBq kg⁻¹ was added dropwise to the soil. The microbial

partitioning into anabolic (i.e., cell growth and maintenance) and catabolic processes (i.e., respiration) was investigated. Immediately after ^{14}C -glucose addition, a polypropylene vial containing 1 ml of 1 M NaOH was placed above the soil to trap the evolved $^{14}\text{CO}_2$. The tubes were then sealed and incubated at 20 °C in the dark to reflect field conditions. The alkaline traps were changed after 1, 3, 6, 10, and 24 h, and then 2, 3, 5, 7, 9, 12, 15, 19, 24 and 30 d after ^{14}C addition. The amount of $^{14}\text{CO}_2$ in the NaOH traps was determined using Optiphase HiSafe 3 scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK).

To determine how much ^{14}C -glucose remained in the soil at the end of the incubation, the soils were extracted with 0.5 M K_2SO_4 at 1:5 (w/v) as described above (Glanville et al., 2016). The extracts were then centrifuged (14,000 g, 15 min) and the supernatant recovered for scintillation counting as previously described for $^{14}\text{CO}_2$ determination. The remaining K_2SO_4 extract was used to determine the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentration in the soil as described earlier. Microbial immobilization of ^{14}C -glucose ($^{14}\text{C}_{\text{imm}}$) at the end of the incubation was estimated as follows:

$$^{14}\text{C}_{\text{imm}} = ^{14}\text{C}_{\text{tot}} - ^{14}\text{C}_{\text{K}_2\text{SO}_4} - ^{14}\text{CO}_2 \quad (\text{Eqn. 5})$$

where $^{14}\text{C}_{\text{tot}}$ is the total amount of ^{14}C -glucose added to the soil at time-zero, $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ is the amount of ^{14}C recovered in the 0.5 M K_2SO_4 extract, and $^{14}\text{CO}_2$ is the total amount of ^{14}C recovered as $^{14}\text{CO}_2$ evolved from the microbial biomass.

Microbial CUE for ^{14}C -glucose was estimated through an exponential kinetic model as described in Glanville et al. (2016) and Jones et al. (2018c). A double first order exponential decay equation was fitted to the experimental data using SigmaPlot v12.5 (Systat Software Ltd., London, UK):

$$^{14}\text{C}_{\text{soil}} = (\text{Mic}_{\text{catab}} \times \exp^{-k_c \cdot t}) + (\text{Mic}_{\text{anab}} \times \exp^{-k_a \cdot t}) \quad (\text{Eqn. 6})$$

where $^{14}\text{C}_{\text{soil}}$ is the amount of ^{14}C remaining in the soil over time (t), $\text{Mic}_{\text{catab}}$ and Mic_{anab} describe the amount of ^{14}C partitioned into the catabolic and anabolic C pools and k_c and k_a correspond to the exponential decay constants describing the turnover of the $\text{Mic}_{\text{catab}}$ and Mic_{anab} pools, respectively. The first pool ($\text{Mic}_{\text{catab}}$) corresponds to a C pool which is rapidly used for catabolic processes, while the second pool (Mic_{anab}) constitutes the remaining C immobilized within the microbial biomass (i.e., used for cell

growth and maintenance, and ultimately necromass turnover) (Glanville et al., 2016). From this, microbial CUE (MiC_{CUE}) can be calculated as follows (Jones et al., 2018c):

$$MiC_{CUE} = MiC_{anab} / (MiC_{catab} + MiC_{anab}) \quad (\text{Eqn. 7})$$

The half-life ($t_{1/2}$) for the first mineralizable pool (MiC_{catab}) can be calculated from:

$$t_{1/2} = \ln(2) / k_c \quad (\text{Eqn. 8})$$

2.2.5 Microbial ^{14}C -labeled arginine mineralization and substrate C use efficiency

Arginine was used as a model substrate to study both C and N mineralization. The very low C:N ratio of arginine (1.5:1) is much lower than the microbial biomass (C:N 7:1) which means that the use of arginine as a sole C source leads to the excretion of excess NH_4^+ from the microbial biomass (Bonde et al., 2001; Fujii et al., 2018). Briefly, 5 g of soil was placed in 50 cm^3 polypropylene tubes to which 0.25 ml of ^{14}C -labeled arginine was added to the soil surface (50 mM; 3.35 kBq ml^{-1} ; 140 mg N kg^{-1} soil, 180 mg C kg^{-1} ; American Radiolabeled Chemicals, St Louis, MO, USA). A polypropylene vial containing 1 ml of 1 M NaOH was placed above the soil to trap any emitted $^{14}\text{CO}_2$, and the tubes were sealed and incubated at 20 °C. The alkaline traps were changed after 1, 3, 6, 9, 24 and 48 h following ^{14}C -substrate addition. The amount of $^{14}\text{CO}_2$ in the NaOH traps was determined as stated before. The short incubation time was chosen to ensure that the substrate was not fully depleted (Jones et al., 2004). At the end of the incubation period (48 h), the amount of ^{14}C -arginine sorbed on the solid phase or in soil solution was extracted with 0.5 M K_2SO_4 solution as described above. The supernatant was then recovered for scintillation counting as for the $^{14}\text{CO}_2$ determination. Microbial immobilization of the arginine-derived ^{14}C ($^{14}\text{C}_{\text{imm}}$) was estimated using Eqn. 1. As there were insufficient data points to fit a double exponential kinetic model, Microbial CUE for ^{14}C -arginine was estimated as follows (Jones et al., 2018c):

$$MiC_{CUE} = ^{14}\text{C}_{\text{imm}} / (^{14}\text{C}_{\text{imm}} + ^{14}\text{CO}_2) \quad (\text{Eqn. 9})$$

The contents of NH_4^+ -N and NO_3^- -N in the soil at the start (0 h) and end (48 h) of the incubation period were determined using 0.5 M K_2SO_4 extracts as described

previously. Ammonification rates of arginine were calculated on a basis of net N accumulation over the incubation period relative to unamended control samples.

2.2.6 Statistical analysis

All procedures were performed in R (version 3.6.3, The R Foundation for Statistical Computing, Boston, MA, USA) and RStudio (v1.2.5019, RStudio, Vienna, Austria). The normality and homoscedastic for the data of the experiments were analysed by Shapiro-Wilk and Bartlett tests. Non-normally distributed data were $\log_{10}$ -transformed prior to analysis (i.e., EC, OC, IN, MBC, DON, and net NH_4^+ -N and NO_3^- -N following arginine addition). Given the soil sampling at two layers (topsoil and subsoil) alongside the effect of correctives and N fertilizer, a three-way ANOVA (correctives $\times$ fertilizer $\times$ soil depth) using a generalized linear model was carried out to assess selected soil properties and parameters derived from soil incubation with ^{14}C -glucose and ^{14}C -arginine. Tukey's HSD post-hoc test was used to compare least-square means through the "emmeans" R package (Lenth et al., 2021). Statistical significance is reported at $P < 0.050$ unless otherwise stated.

2.3 RESULTS

2.3.1 Soil characteristics

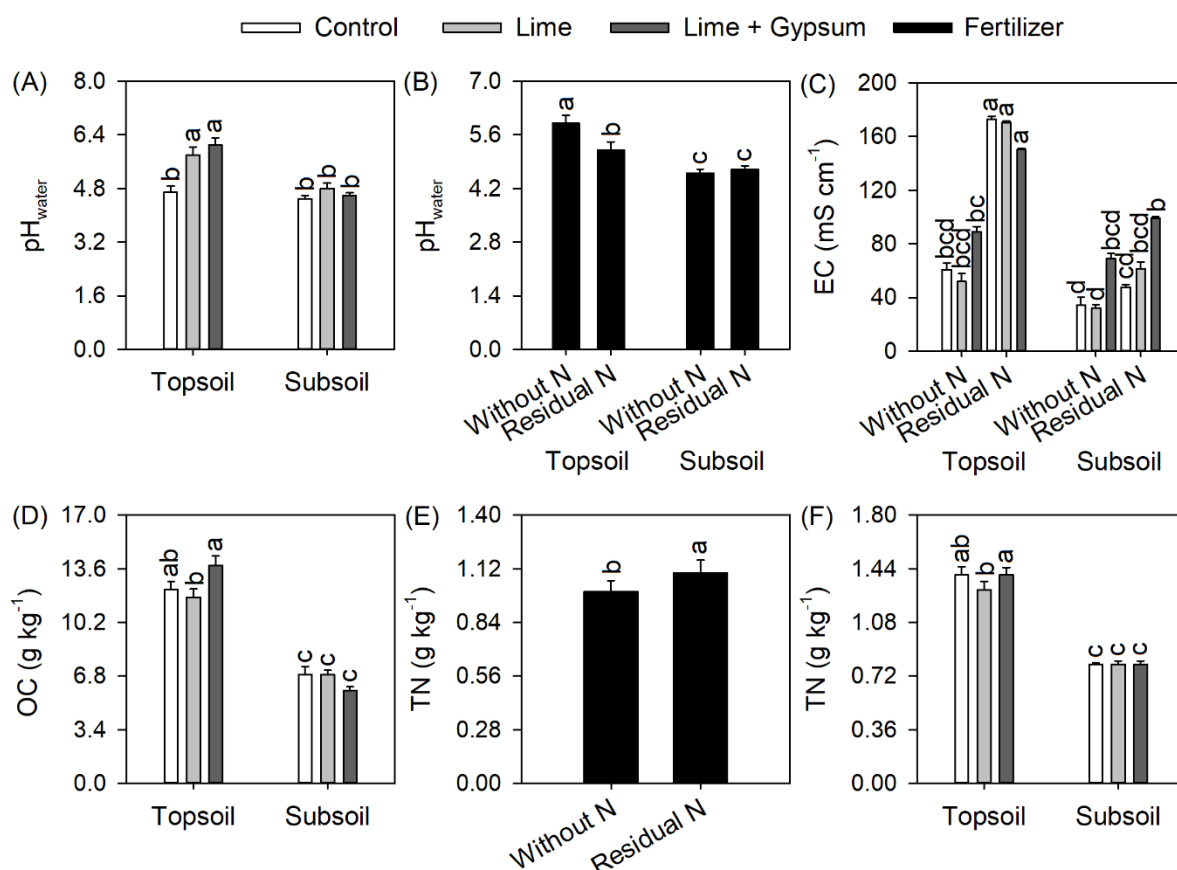
The pH had an interaction between corrective and soil layer, as well as between N fertilizer and soil layer (Table 1). In particular, pH in the amended treatments was ~ 1.2 unit greater than the control for the topsoil only (Fig 1A). Further, control treatment was similar in both the topsoil and subsoil and also similar to those treatments that received lime and lime + gypsum in the subsoil (Fig 1A). In addition, residual N decreased 0.7 unit of the pH in the topsoil, while in the subsoil, with the lowest values, there was no effect of N fertilizer (Fig 1B). Electrical conductivity had a three-way interaction (Table 1). All corrective treatments with residual N in the topsoil had the highest EC values (173, 171, and $150 \mu\text{S cm}^{-1}$, respectively; Fig 1C). The application of lime + gypsum with N addition increased the EC in the subsoil relative to the unamended control (regardless of the N management) and lime without N (Fig 1C).

Table 1 - Probability (*P*) values associated with the following factors: corrective (control, lime, and lime + gypsum); N fertilizer (without N and residual N); and soil layer (topsoil and subsoil) for soil properties and parameters obtained from the laboratory assays using ^{14}C -labeled tracers. *P*-values ≤ 0.050 are highlighted in bold

Variable	Factor						
	Corrective	Fertilizer	Layer	C×F	C×L	F×L	C×F×L
Soil							
pH _{water}	<0.001	0.010	<0.001	0.939	0.001	0.002	0.977
EC	0.003	<0.001	<0.001	0.150	0.021	<0.001	0.047
OC	0.493	0.906	<0.001	0.526	0.006	0.142	0.940
TN	0.125	0.002	<0.001	0.315	0.042	0.144	0.518
IN	0.598	<0.001	<0.001	0.113	0.020	0.011	0.002
DOC	0.314	<0.001	0.144	0.122	0.039	0.579	0.001
DON	<0.001	0.003	<0.001	0.048	<0.001	0.878	0.029
MBC	<0.001	<0.001	<0.001	0.104	0.380	0.723	0.001
MBN	0.045	0.016	<0.001	0.405	0.367	0.002	0.371
^{14}C-							
$^{14}\text{CO}_2$	0.008	0.198	0.948	0.144	0.819	0.371	0.431
$^{14}\text{C}_{\text{K}_2\text{SO}_4}$	0.002	0.099	<0.001	0.320	0.009	0.340	0.244
$^{14}\text{C}_{\text{immob}}$	0.006	0.183	0.184	0.113	0.918	0.349	0.417
Mic _{CUE}	0.360	0.618	0.569	0.502	0.575	0.769	0.340
Mic _{catab} $t_{1/2}$	<0.001	0.275	<0.001	<0.001	0.012	0.081	0.003
^{14}C-							
$^{14}\text{CO}_2$	0.089	0.426	<0.001	0.161	0.168	0.052	0.066
$^{14}\text{C}_{\text{K}_2\text{SO}_4}$	<0.001	0.003	<0.001	0.012	0.002	0.001	0.016
$^{14}\text{C}_{\text{immob}}$	0.022	0.051	<0.001	0.093	0.356	0.369	0.063
Mic _{CUE}	0.208	0.971	<0.001	0.278	0.589	0.334	0.079
$^{14}\text{CO}_2:\text{NH}_4^+-$	<0.001	<0.001	<0.001	<0.001	0.424	0.002	0.004
Net NH_4^+-N	<0.001	0.010	<0.001	0.003	<0.001	0.400	0.695
Net NO_3^--N	0.657	<0.001	<0.001	0.036	0.180	0.001	0.004

pH: pH in water; EC: electrical conductivity; OC: organic C; TN: total N; IN: inorganic N; DOC: dissolved organic C; DON: dissolved organic N; MBC: microbial biomass C; MBN: microbial biomass N; $^{14}\text{CO}_2$: $^{14}\text{CO}_2$ evolved from the soil; $^{14}\text{C}_{\text{K}_2\text{SO}_4}$: ^{14}C recovered in the soil solution; $^{14}\text{C}_{\text{immob}}$: ^{14}C immobilized into the microbial biomass; Mic_{CUE}: microbial C use-efficiency of the substrate; Mic_{catab} $t_{1/2}$: half-life of the first mineralizable pool; $^{14}\text{CO}_2:\text{NH}_4^+-\text{N}$: the ratio between $^{14}\text{CO}_2$ evolved and NH_4^+-N in the soil at the end of the incubation; Net NH_4^+-N : net NH_4^+-N production at the end of the incubation; Net NO_3^--N : net NO_3^--N production at the end of the incubation.

Figure 1 - Chemical soil properties measured before the incubation assays. pH affected by corrective $\times$ layer and fertilizer $\times$ layer interactions (A and B, respectively); electrical conductivity (EC) affected by a corrective $\times$ fertilizer $\times$ layer interaction (C); organic C (OC) affected by a corrective $\times$ layer interaction (D); total N (TN) affected by the main effect of fertilizer and a corrective $\times$ layer interaction (E and F, respectively). Only significant effects (Table 1) were shown. The error bars represent the standard error of the mean. Common letters do not indicate differences by the Tukey's HSD test at $P \leq 0.05$. SOURCE: the author.

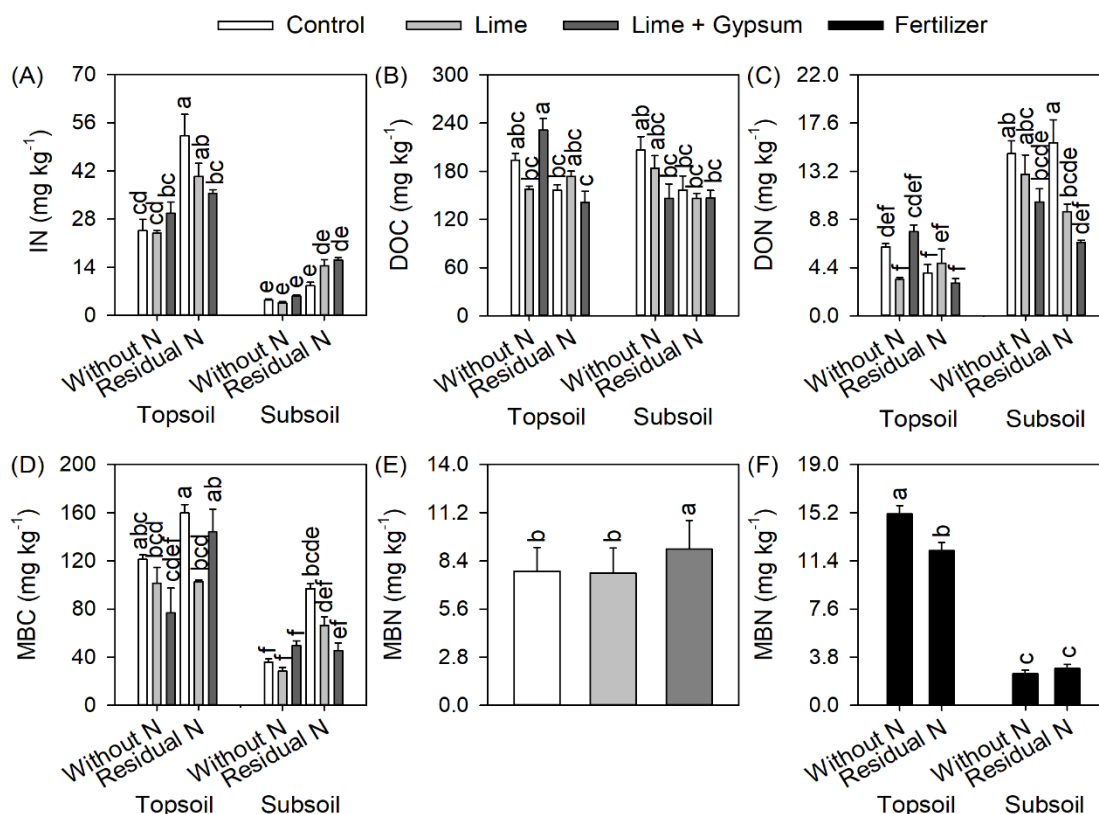


Organic C had an interaction between corrective and soil layer (Table 1). In the topsoil, the OC content was 14% higher for the lime + gypsum treatment compared with lime alone, although there was no difference between them and the control (Fig 1D). On average, the topsoil had an increase of 6.0 g kg^{-1} in the OC content compared with the subsoil, while correctives had no effect in the latter layer (Fig 1D). Total N was affected by the main effect of fertilizer (Table 1, Fig 1E), with higher content under residual N over without N addition. Similar to OC, TN content also had a corrective $\times$ soil layer interaction (Table 1), with higher content in the lime + gypsum compared with lime in the topsoil (Fig. 1F). Moreover, the topsoil had, on average, a TN content 0.60

g kg⁻¹ higher than the subsoil, which had no difference among corrective treatments (Fig 1F).

Inorganic N, DOC, DON, and MBC had a three-way interaction (Table 1). Inorganic N was higher in the control with residual N compared with the other treatments in the topsoil, except lime with N addition (Fig. 2A). There were no differences among treatments in the subsoil for IN, in addition to the overall lower content in this layer relative to the topsoil (Fig. 2A). For DOC, the treatments lime + gypsum without N in the topsoil and control without N in the subsoil were higher than lime + gypsum with residual N in the topsoil (Fig. 2B). Overall, the DOC content in the topsoil and subsoil were similar (Fig. 2B). The control (regardless of the N management) in the subsoil had higher DON content compared with all topsoil treatments (which had overall lowest values) and lime + gypsum with residual N in the subsoil (Fig. 2C). The MBC content was higher in the control and lime + gypsum with residual N in the topsoil in comparison with lime + gypsum without N in the same layer and all treatments in the subsoil, with exception to control with N (Fig. 2D). Microbial biomass-N presented the main effect of corrective (Table 1), where lime + gypsum was 15% higher, on average, compared with lime alone and unamended control (Fig. 2E). In addition, a fertilizer × layer interaction was also found (Table 1), where MBN content was higher without N relative to residual N addition in the topsoil, while the subsoil had the lowest values and was not affected by N management (Fig. 2F).

Figure 2 - Chemical soil properties measured before the incubation assays. Inorganic N (IN), dissolved organic (DOC), dissolved organic N (DON), and microbial biomass C (MBC) affected by a corrective × fertilizer × layer interaction (A, B, C, and D, respectively); and microbial biomass N (MBN) affected by the main effect of corrective and by a fertilizer × layer interaction (E and F, respectively). Only significant effects (Table 5) were shown. The error bars represent the standard error of the mean. Common letters do not indicate differences by the Tukey's HSD test at $P \leq 0.05$.



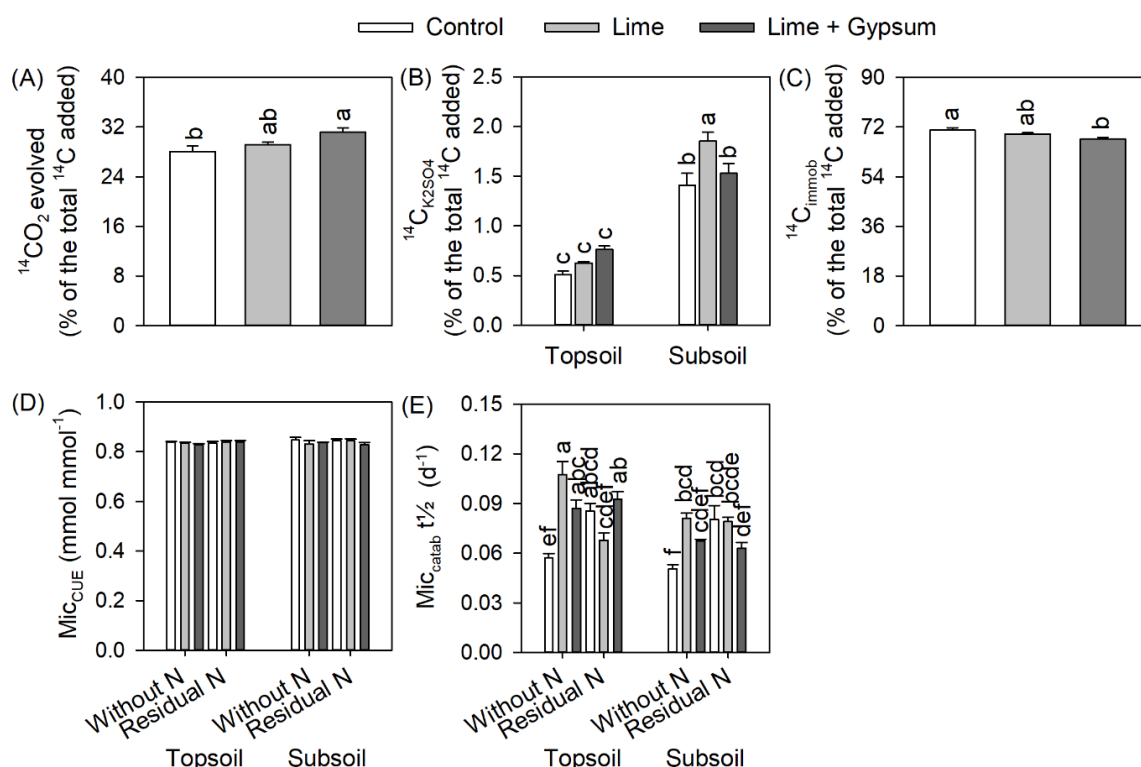
SOURCE: the author.

2.3.2 Microbial C-use efficiency with a glucose substrate

The main effect of corrective was observed for $^{14}\text{CO}_2$ evolved from the soil (Table 1), which had the following trend: lime + gypsum > lime > control, where 28–31% of the total ^{14}C -labeled glucose added to the soil was respired by microbes (Fig. 3A). In addition, less than 2% of the glucose-derived ^{14}C was recovered in the K_2SO_4 solution (*i.e.*, the proportion of ^{14}C sorbed to the solid phase or available in the soil solution) at the end of the incubation period, with a higher amount observed in the subsoil than the topsoil for the corrective × layer interaction (Table 1; Fig. 3B). While no differences were observed in the topsoil, the lime-treated soil had the highest value of $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ in the deep layer (Fig. 3B). The $^{14}\text{C}_{\text{immob}}$ was influenced by the main effect

of corrective (Table 1) similarly to the $^{14}\text{CO}_2$ evolved, but with an opposite trend, where a higher amount of ^{14}C was immobilized by microbes in the control in comparison with the lime + gypsum treatment (Fig. 3C). Despite the above results, Mic_{CUE} had no effect of corrective, N fertilizer, nor soil layer, therefore averaging $0.84 \text{ mmol mmol}^{-1}$ (Fig. 3D). Moreover, a low amount (16%, representing the mean value across all treatments) of added ^{14}C –glucose was rapidly respired by soil microbes (*i.e.*, $\text{Mic}_{\text{catab}}$ pool), while the majority of the ^{14}C (82%, mean value across all treatments) was incorporated into the microbial biomass (*i.e.*, Mic_{anab} pool) before its partial mineralization as CO_2 (data not shown). However, $\text{Mic}_{\text{catab}} t_{1/2}$ was influenced by a three–way interaction (Table 1). In this regard, the unamended control without N addition in the subsoil had a lower $\text{Mic}_{\text{catab}} t_{1/2}$ compared with the other treatments, except control without N and lime with N in the topsoil, as well as lime + gypsum with N in the subsoil (Fig. 3E).

Figure 3 - Microbial and chemical parameters obtained at the end (30 d) of the ^{14}C -labeled glucose assay. $^{14}\text{CO}_2$ evolved from the soil affected by the main effect of corrective (A); ^{14}C recovered in the soil solution ($^{14}\text{CK}_2\text{SO}_4$) affected by a corrective $\times$ layer interaction (B); ^{14}C immobilized into the microbial biomass ($^{14}\text{C}_{\text{immob}}$) affected by the main effect of corrective (C); and half-life of the first mineralizable pool ($\text{Mic}_{\text{catab}} t_{1/2}$) affected by a corrective $\times$ fertilizer $\times$ layer interaction (E). Only significant effects (Table 1) were shown, except for the microbial C use-efficiency of the substrate (Mic_{CUE}), where no effect was observed but all treatments were displayed (D). The error bars represent the standard error of the mean. Common letters do not indicate differences by the Tukey's HSD test at $P \leq 0.05$.



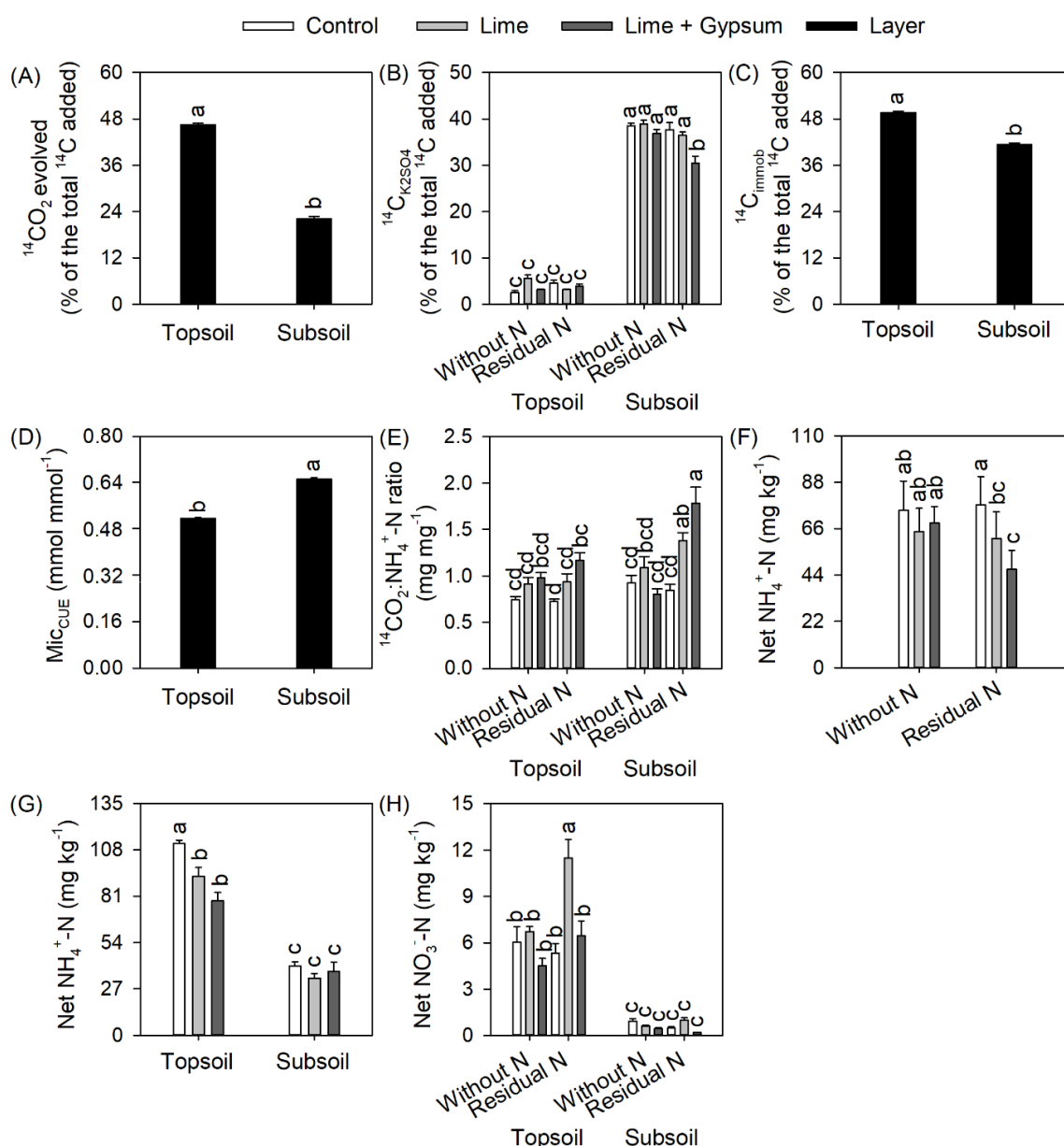
SOURCE: the author.

2.3.3 Microbial ^{14}C -labeled arginine mineralization and substrate C use-efficiency

The main effect of soil layer influenced the ^{14}C -arginine mineralization (Table 1), which was much higher in the topsoil than in the subsoil, where 47% and 22% of the total ^{14}C added was evolved as CO_2 after 48 h, respectively (Fig. 4A). The amount of arginine-derived ^{14}C recovered in the K_2SO_4 solution had a corrective $\times$ fertilizer $\times$ layer interaction (Table 1). Overall, 32% more $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ was recovered from the subsoil in comparison to the topsoil solution at the end of the incubation period (Fig. 4B). While all treatments were similar in the topsoil, lime + gypsum with residual N was the lowest across the subsoil (Fig. 4B). In addition, both $^{14}\text{C}_{\text{immob}}$ and Mic_{CUE} were influenced by

the main effect of layer, similar to $^{14}\text{CO}_2$ (Table 1). Thus, a higher amount (22%) of ^{14}C –arginine was immobilized by microbes in the topsoil than the subsoil (Fig. 4C), while Mic_{CUE} was 25% higher in the deep layer than in the topsoil (Fig. 4D). The $^{14}\text{CO}_2:\text{NH}_4^+-\text{N}$ ratio, which could be used to decouple C and N utilization and mineralization of N compounds (arginine, in this case), was affected by a three–way interaction (Table 1), where lime + gypsum with N in the subsoil had a higher ratio in comparison with the remaining treatments, except lime alone with residual N in the same layer (Fig. 4E). With regard to the net NH_4^+-N in the soil following arginine addition, two interactions were observed, as follows: corrective $\times$ fertilizer and corrective $\times$ layer (Table 1). Under residual N addition, NH_4^+-N content in the control was higher compared with lime and lime + gypsum, while treatments without N were similar between them (Fig. 4F). Without any difference among treatments, the subsoil had lower net NH_4^+-N content relative to the topsoil, which presented the following trend: control > lime $\approx$ lime + gypsum, with values in the range of 78–111 mg kg $^{-1}$ (Fig. 4G). Lastly, the net NO_3^--N was affected by a three–way interaction (Table 1). With a pattern comparable to the NH_4^+-N dynamics, the subsoil had much lower NO_3^--N in comparison with the surface layer (mean value of 0.61 and 6.77 mg kg $^{-1}$, respectively), in addition to the lack of difference among treatments (Fig. 4H). In the topsoil, however, the lime–treated soil with residual N presented the highest NO_3^--N content, while the remaining treatments did not differ from each other (Fig. 4H).

Figure 4 - Microbial and chemical parameters obtained at the end (48 h) of the ^{14}C -labeled arginine assay. $^{14}\text{CO}_2$ evolved from the soil affected by the main effect of layer (A); ^{14}C recovered in the soil solution ($^{14}\text{C}_{\text{K}_2\text{SO}_4}$) affected by a corrective $\times$ fertilizer $\times$ layer interaction (B); ^{14}C immobilized into the microbial biomass ($^{14}\text{C}_{\text{immob}}$) and microbial C use-efficiency of the substrate (Mic_{CUE}) affected by the main effect of layer (C and D, respectively); the ratio between $^{14}\text{CO}_2$ evolved and $\text{NH}_4^+\text{-N}$ in the soil ($^{14}\text{CO}_2:\text{NH}_4^+\text{-N}$) affected by a corrective $\times$ fertilizer $\times$ layer interaction (E); net $\text{NH}_4^+\text{-N}$ production affected by corrective $\times$ fertilizer and corrective $\times$ layer interactions (F and G, respectively); and net $\text{NO}_3^-\text{-N}$ production affected by a corrective $\times$ fertilizer $\times$ layer interaction (H). Only significant effects (Table 1) were shown. The error bars represent the standard error of the mean. Common letters do not indicate differences by the Tukey's HSD test at $P \leq 0.05$.



SOURCE: the author.

2.4 DISCUSSION

2.4.1 *Influence of lime and gypsum amendment on soil properties*

The increase in soil pH in the topsoil following two years of lime application was similar to the response reported by Inagaki et al. (2017), who found a decrease in the soil acidity in the topsoil but not in the subsoil, with no effect of gypsum. Garbuio et al. (2011) also observed a relative increase in soil pH down to 20 cm in a no-till system, 30 mo after lime addition. The addition of N fertilizer also lowered topsoil pH, which we attribute to the application of NH_4^+ -based fertilizer to maize, similar to the findings of Garbuio et al. (2011). Although the application of lime, gypsum, and N fertilizer did not change subsoil pH, we did observe an increase in EC for the treatment with lime + gypsum coupled to residual N compared with control without N and lime, irrespective of the N management. We attribute this to the addition of gypsum, which promotes the vertical displacement of ions to deep horizons (Ritchey et al., 1980), including those derived from the N fertilizer.

Organic C and TN concentrations were higher in the topsoil relative to the subsoil. We ascribe this to the higher root mass in this layer and the deposition of plant residues on the soil surface. While C and N inputs on topsoil are derived from shoot residues and fertilizer addition, subsoil inputs are largely root-derived and tend to be less labile, and therefore more resilient (Jones et al., 2018b; Struecker et al., 2016). Liming is frequently associated with soil C losses through CO_3^{2-} dissociation (Kemmitt et al., 2006; Pachauri et al., 2014), stimulation of soil microbial activity, and solubilization of organic matter (Carmeis Filho et al., 2017; Inagaki et al., 2017; Vieira Fontoura et al., 2019). However, adequate management of pH and base cation correctives have the potential to enhance C storage in soils, directly through enhanced plant growth or indirectly by reducing soil erosion (Carmeis Filho et al., 2017; Inagaki et al., 2016). Although our study had higher OC in the topsoil in the lime + gypsum treatment compared with lime applied alone, they did not differ relative to the control. As observed here, the application of lime $\pm$ gypsum has previously been shown to have minimal effect on TN concentration, at least in the short-term, showing a similar trend as the corrective $\times$ layer interaction for OC (Garbuio et al., 2011; Inagaki et al., 2017). However, an increase in TN stocks could be obtained over longer timescales, with the increase in deposition of organic materials due to the tight stoichiometric balancing of

C and N in SOM (Carmeis Filho et al., 2017). In addition, we suggest that the highest TN concentration with residual N fertilization can be explained by a higher biomass return to the soil (Diekow et al., 2005).

Inorganic N in the soil increased with N fertilizer application, but only in the topsoil. Similar results were observed by Garbuio et al. (2011), which reported higher NO_3^- -N in the top 10-cm, and no difference for NH_4^+ -N among treatments with inorganic fertilizer application. Further, Garbuio et al. (2011) observed no effect of liming on IN concentration for bare soil compared with fallow. Andersson et al. (2000) reported a rise in soil pH, as well as increased microbial activity (measured as CO_2 respiration), increased DOC and DON leaching in lime-treated mor humus. This was not the case here since DOC and DON concentrations did not increase in the subsoil in response to higher soil pH in topsoil (*i.e.* lime and lime + gypsum treatments). Contrary to Jones et al. (2018b), who found decreased DON concentration and increasing DOC-to-DON ratio with depth, while DOC concentration did not present great differences between treatments nor soil layer, we observed that DON concentration was overall higher in the subsoil than in the topsoil. The lower concentration of DON in the surface could be attributed to the greater root density and therefore to higher plant uptake in the topsoil since Guinea grass was growing in the field during soil sampling for this study. In addition, the difference in DON concentration among soil layers could be attributed to the higher microbial activity related to the N cycle, as observed in the ^{14}C -arginine assay, and the rapid microbial removal of labile DON from solution as suggested by Jones et al. (2018b).

Similar to OC and TN concentrations, MBC and MBN were higher in the topsoil compared with the subsoil (Jones et al., 2018b). In addition, a positive effect of N fertilization on MBC was observed in the subsoil for the control treatment. Aye et al. (2018) reported a positive effect of N addition on MBN, but the effect on MBC was suppressive, while Wachendorf (2015) observed an increase in both MBC and MBN with N application to soil without root litter. Liming and high soil pH also generally have positive effects on MBC and MBN, with a subsequent higher microbial activity (Aye et al., 2018; Kemmitt et al., 2006). However, under residual N, lime had lower MBC compared with control treatment in the topsoil, while similar MBN was obtained in control and lime applied alone (main effect of corrective). These findings are in line with Wachendorf (2015), who observed a decrease in MBC following lime addition. Liming can induce a short-term increase in the available C substrate, often increasing

microbial activity, but microbial biomass may not change or even decrease due to the progressive depletion of these substrates (Badalucco et al., 1992; Wachendorf, 2015).

2.4.2 C partitioning and microbial C use efficiency in response to glucose addition

The addition of a low concentration of ^{14}C –glucose allowed us to mimic natural rates of C turnover in the soil (de Sosa et al., 2018; Glanville et al., 2016; Jones et al., 2018b). The $^{14}\text{CO}_2$ emission and kinetic parameters from the decay equation (data not shown) did not exhibit any major difference in ^{14}C processing between the topsoil and subsoil following glucose addition, even with a higher MBC and MBN in the topsoil. The activity of soil microbial communities and labile C availability are often considered the main drivers of soil respiration (Glanville et al., 2016). However, a slight difference in the ^{14}C –glucose turnover (*i.e.*, $^{14}\text{CO}_2$ evolved and $^{14}\text{C}_{\text{immob}}$) was observed in response to lime and gypsum application. This suggests that the microbial community structure has not changed greatly in response to soil correctives, or if they have changed, metabolic pathways were preserved. Aye et al. (2018), applying a large amount of ^{13}C –glucose ($500 \mu\text{g C g}^{-1}$ soil) observed 7% less substrate–derived CO_2 from a strongly acid (pH of 4.1) in comparison with a slightly acid soil (pH of 6.6). Moreover, Kemmitt et al. (2006) reported no difference in the mineralization as of ^{14}C –glucose for soil with different pH values (3.5–6.8). Our findings support the above are congruent with the above studies. In addition, the overall higher $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ in the subsoil is likely explained by the lower microbial activity in this soil layer relative to the topsoil, where MBC and MBN are higher.

The Mic_{CUE} for ^{14}C –glucose averaged 0.84, which is considered normal to high since the average value for sugars in the soil is 0.68, but ranging from 0.25 to 0.95 (Jones et al., 2018a; Jones et al., 2019). Comparing 970 agricultural soils from Australia, Jones et al. (2019) observed a progressive decline in Mic_{CUE} with increasing soil acidity at pH values below 5.5. Despite the range of soil pH values found here (from 4.4 to 6.5), which includes the transition point at which Mic_{CUE} declined [*i.e.*, pH of 5.5, as reported by Jones et al. (2019)], we found no difference among treatments. In general, the ^{14}C –glucose presented a rapid turnover, with $\text{Mic}_{\text{Catab}} t_{1/2} < 0.5 \text{ d}$, suggesting a slightly high C flux through the soil solution, in comparison to the subsequent flux of substrate–derived C through the biomass (Glanville et al., 2016). However, the fastest turnover was obtained by the control, regardless of the N

management (Mic_{catab} $t_{1/2}$ of 0.057 d (or 1.3 h) in the topsoil and 0.051 d (or 1.2 h) in the subsoil, suggesting that catabolic processes were slower in the absence of soil correctives. This can be a result of indirect acidity stress such as Al toxicity, which is known to interfere with some respiratory pathways, requiring a re-routing of the metabolism and leading into more inefficient C pathways (Jones et al., 2019).

2.4.3 C partitioning and microbial C use efficiency in response to arginine addition

Unlike ^{14}C –glucose, the turnover of ^{14}C –arginine was different between the two layers, with higher $^{14}\text{CO}_2$ respired and overall much lower amounts of ^{14}C – K_2SO_4 in the topsoil. In general, different substrates lead to different Mic_{CUE} values (Jones et al., 2018a), with a higher Mic_{CUE} for sugars relative to amino acids (~ 0.68 and ~ 0.55 , respectively). Jones et al. (2018b) reported a decrease in $^{14}\text{CO}_2$ evolution in the first 6 h across soil depths, but mineralization rates were similar after 48 h. Garbuio et al. (2011) observed higher $^{14}\text{CO}_2$ evolved in surface soil up to 24 h following ^{14}C –amino acid mix (containing equimolar proportions to give an individual amino acid concentration of $666 \mu\text{mol L}^{-1}$ and specific activity of 1.0 kBq mL^{-1} : alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine) addition in lime-treated soils. However, after 48 h, there was no difference for arginine-derived $^{14}\text{CO}_2$ between soil correctives nor fertilizer management, regardless of the soil layer. Short-term incubation assay of arginine (from 24 h to 96 h) is generally used to provide information on enzyme (e.g., arginase) activity in microbial communities and is strongly influenced by soil C:N ratios (Fujii et al., 2019). On temperate acid-forests, Fujii et al. (2020) observed a decrease in arginine mineralization (as $^{14}\text{CO}_2$ evolution) with increasing soil C:N ratio ($\text{C:N} > 20$), and a greater flux with increasing soil pH. However, C:N ratio was beyond values pointed by Fujii et al. (2020) for both soil layers (9.4 in the topsoil and 8.3 in the subsoil), and the $^{14}\text{CO}_2$ evolution from arginine was higher in the topsoil, where the C:N ratio was higher. In contrast to Fujii et al. (2020) and Jones et al. (2018b), Bonde et al. (2001) reported a positive correlation of arginine ammonification with microbial biomass and microbial activity. Evaluating the long-term effect of lime and N fertilizer application on amino acid mix (standard amino acid mix, of 333 mM and specific activity of 1.3 kBq mL^{-1} : alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine,

tyrosine, and valine; final concentration 5 mM) turnover in agricultural soils, Jones et al. (2005) observed no effect on $^{14}\text{CO}_2$ evolved following N fertilizer application, while soil pH slightly decreased. The authors concluded that total microbial activity is likely a key determinant of amino acid turnover since management of agricultural soils (e.g., the effect of N, P, and K fertilizer addition; grazing; pH manipulation with lime addition; vegetation cover and shifts comparing grassland versus arable; and drainage) have shown little effect on topsoil. Therefore, the higher MBC and MBN in the topsoil could be related to ^{14}C –arginine turnover response.

The Mic_{CUE} for ^{14}C –arginine had an opposite trend from its turnover, with the highest value verified in the subsoil. Kemmitt et al. (2006) reported lower amino acid turnover with deep soil horizons, reflecting the higher microbial activity found in upper layers. Therefore, the higher Mic_{CUE} in the subsoil is likely explained by the lower microbial biomass turnover in this layer, which is supported by the higher amount of ^{14}C –arginine immobilized and much lower $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ recovered in the topsoil than in the subsoil. However, the $^{14}\text{CO}_2:\text{NH}_4^+-\text{N}$ ratio was similar between the topsoil and subsoil. The treatment lime + gypsum with residual N had the lowest efficiency regarding ^{14}C –arginine mineralization, with a higher proportion of $^{14}\text{CO}_2$ evolved per molecule of NH_4^+-N mineralized. Evaluating the selective N mineralization relative to C from arginine, Fujii et al. (2020) reported $^{14}\text{CO}_2:\text{NH}_4^+-\text{N}$ ratios varying from 1.0 to 1.5 after 96 h incubation, which is similar to our range (0.74–1.78) for a 48–h incubation period. Moreover, Fujii et al., 2020 suggest that C and N mineralization was decoupled for treatments with the highest $^{14}\text{CO}_2:\text{NH}_4^+-\text{N}$ ratio (1.5), indicating differences in intercellular activities in the arginine degradation into urea ($\text{CH}_4\text{N}_2\text{O}$) and ornithine ($\text{C}_5\text{H}_{12}\text{N}_2\text{O}_2$). Since there was no difference for Mic_{CUE} of ^{14}C –arginine related to corrective nor fertilizer, the difference presented for lime + gypsum with residual N could be related to repressed degrading enzyme activity and/or internal N preservation in fungal biomass by the re-utilization of ornithine (Fujii et al., 2020).

Despite the lower net NH_4^+-N production with lime and lime + gypsum compared with the control in the topsoil, the highest net NO_3^--N content occurred for lime with residual N fertilizer in the same layer. Our results partly agree with Garbuio et al. (2011), who observed higher N mineralization and nitrification under liming. A positive correlation between soil pH and arginine ammonification was also obtained by Fujii et al. (2020). In addition, Garbuio et al. (2011) observed that N fertilizer application did not affect the ^{14}C mineralization of the amino acid mix, but enhanced nitrification

rates with increasing soil depth. In general, increased availability of inorganic N does not repress microbial amino acid mineralization in short-term laboratory incubation due to the fast nitrification in agricultural soils (Jones et al., 2005). Furthermore, the higher $^{14}\text{C}_{\text{K}_2\text{SO}_4}$ amount and the lower net production of mineral N in the subsoil compared with the topsoil suggests a slower arginine degradation in the deep layer. In addition, Jones et al. (2018b) reported higher net $\text{NH}_4^+\text{--N}$ production following arginine addition, suggesting that the subsoil microbial activity is more C-limited rather than by N. Although the lower arginine mineralization in the subsoil, DON concentration was high in this layer, suggesting an effect of repressed enzyme activity (Fujii et al., 2020) since DON compounds are preferred N sources by microorganisms in comparison to NO_3^- (Abaas et al., 2012; Jones et al., 2018b)

2.5 CONCLUSIONS

The surface application of lime and gypsum only alleviates soil acidity in the topsoil. However, despite a slight increase of $^{14}\text{CO}_2$ evolution of added glucose, the Mic_{CUE} of treatments with corrective is similar to the unamended control, regardless of the N fertilization and soil layer. Apart from the difference in soil properties among layers, $^{14}\text{CO}_2$ evolved from glucose suggests that both topsoil and subsoil microbial biomass are similarly active. On the other hand, the Mic_{CUE} of ^{14}C –arginine is only affected by the soil layer, where the topsoil is higher than the subsoil, while net $\text{NH}_4^+\text{--N}$ and $\text{NO}_3^-\text{--N}$ productions are affected by the amendments. However, the data indicates that these results are not directly related to soil pH, the main response to lime and/or gypsum, or with the C:N ratio, as previously described in the literature. The N cycling in both topsoil and subsoil is limited by upstream elements in the N cycle (*i.e.*, substrate availability for protease action). Thus, the influence of lime and gypsum seems to be related to nutrient availability, nitrification, microbial activity, and microbial community structure. Further research is needed to unravel the profile of microbial communities, among other soil properties, caused by the application of lime and gypsum and demystify the theory that these correctives are harmful sources of CO_2 to the atmosphere.

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CHAPTER 3

LIME AND PHOSPHOGYPSUM APPLICATION FAVOURS THE ABUNDANCE OF BENEFICIAL FUNGI AND N-CYCLED RELATED BACTERIA

ABSTRACT

Soil microbial communities are the main drivers of C and N cycles in terrestrial ecosystems, and are probably the earliest indicators of soil quality and potential for the mitigation on the climate change. As they are critically affected by soil pH, it is paramount to understand how the soil microbial community is affected by lime and phosphogypsum applications. We evaluated the short-term effects of superficial lime (L) and gypsum (G) application, and N-fertilizer on selected soil properties and microbial communities, via phospholipid fatty acids (PLFA) and Illumina 16S ribosomal RNA (rRNA) amplicon sequencing. Soil pH was the most important characteristic regulating soil microbial structure. Lime and gypsum (L+G) application alleviated acidity stress, and decreased the gram positive to gram negative ratio (Gram+:Gram-), furthermore, the Fungi:Bacteria ratio was increased when PLFA were used as biomarkers for the different taxonomic groups. In addition, arbuscular mycorrhizal fungi (AM Fungi) were enhanced by L+G. Based on amplicon sequencing, Bacteria relative abundance was affected at both family and genus level. In general, L and L+G application favoured the relative abundance of important taxonomic groups involved in the N cycle. In addition, except for bacteria genera involved in N₂ fixation, N-fertilizer application also increased the relative abundance of groups involved in N cycle. We concluded that L and L+G application favour the abundance of microbial groups considered beneficial as AM Fungi and N cycle bacteria genera by alleviating stress acidity. Moreover, despite no changes in the phylum-level, in the short-term, our results assists the capacity to predict the future behaviour of soil microbial communities.

Keywords: Microbial diversity, soil acidity, no-till system, soil conservation, bacterial community

3.1 INTRODUCTION

Reduce greenhouse gases emissions and increase carbon (C) conservation and sequestration are key mitigating factors for climate change (Purwanto and Alam, 2020). Soils are reported to store two thirds of the total terrestrial C, but depending on the management, primarily in intensive agricultural land, they can act as net sink or a net source of C (Lorenz and Lal, 2018). The dynamics of C cycle is linked with the dynamics of nitrogen (N) (Lorenz and Lal, 2018; Purwanto and Alam, 2020). Increasing N inputs have been recognized as an important factor directly influencing belowground C cycling (Delgado-Baquerizo et al., 2018; Rousk et al., 2011). Thus, intensive agricultural systems, when not properly managed, can lead to soil N depletion (Raphael et al., 2016; Rigon and Calonago, 2020; Rocha et al., 2020) and, consequently, to a loss of soil organic matter and soil C, through erosion and leaching process (Purwanto and Alam, 2020; Raphael et al., 2016).

The cycles of C and N in soil are mainly regulated by the soil microbial community (Delgado-Baquerizo et al., 2018), and soil pH is one of the most important driver affecting the microbial structure (Jones et al., 2019; Rousk et al., 2011). Low soil pH critically affects bacteria diversity (Zhalnina et al., 2015), Fungi:Bacteria ratios (Rousk et al., 2010), N mineralization (Aciego Pietri and Brookes, 2008), microbial C use efficiency (CUE) and turnover rates (Jones et al., 2019; Malik et al., 2018), among others. Furthermore, microbial trade-off to soil acidity stress adaptation and biomass production lead to lower potential for C storage through increased microbial growth efficiency (Malik et al., 2018). In addition, the use of N fertilizer can affect the soil microbial biomass (Rousk et al., 2011; Zhalnina et al., 2015), and soil microbial community composition, especially bacteria diversity related with N metabolism (Lammel et al., 2018; Liu et al., 2021; Zhalnina et al., 2015). Moreover, the addition of ammonium-based fertilizer increase soil acidity (Ahmad et al., 2020).

Amelioration of soil acidity can easily be done through liming (Holland et al., 2018; Ritchey et al., 1980). However, the effects of lime application on the soil C stock remain controversial (Fornara et al., 2011; Gibbons et al., 2014; Holland et al., 2018). Despite increasing soil pH and alleviating stress acidity, lime application is associated to increased soil respiration due to a stimulation in microbial activity in addition to the carbonate (CO_3^{2-}) reaction with H^+ (Gibbons et al., 2014; Ritchey et al., 1980).

Accordingly, Abalos et al. (2020) observed higher CO₂ emissions, even after liming promoted a higher carbon use efficiency (CUE). Furthermore, aiming to increase subsoil fertility and reduce Al³⁺ toxicity, gypsum can be applied in combination with lime (Carneis Filho et al., 2017; Inagaki et al., 2017). Although gypsum does not affect soil pH, the addition of gypsum may influence microbial community in topsoil especially in the short-term by providing nutrients (Ca²⁺ and S) (Carneis Filho et al., 2017; Garbuio et al., 2011; Inagaki et al., 2017; Ritchey et al., 1980).

Thus, understanding the main aspects that affect soil microbial community structure and biomass is a potential strategy for the mitigation of climate change (Purwanto and Alam, 2020; Zelles, 1999). Accessing alterations in soil microbial community structure, for example, via phospholipid fatty acid (PLFA) analysis, is commonly used as an indicator of the biological component for soil health assessment (Veum et al., 2019). Conversely, significant advances in the investigation of microbial communities have been made with the use of high-throughput DNA sequencing techniques with superior taxonomic resolution (Sun et al., 2020; Zhalnina et al., 2015). Furthermore, the combination of both techniques are likely to provide much more complete information on the soil microbial structure and functioning (Sun et al., 2020; Zhalnina et al., 2015). In this regard, both methods, PLFA- and DNA-based sequencing (bacterial 16S rRNA), were used to assess the influence of the co-addition of lime, gypsum and N fertilizer in the microbial community structure and bacterial diversity in soil, and to elucidate how their impact on C and N dynamics.

3.2 MATERIAL AND METHODS

3.2.1 Site characteristics and treatments

Soil was collected from a field experiment located in Botucatu, State of São Paulo, Brazil (770 m a.s.l.; 22° 49'51.6" S; 48° 25'38.6" W). The soil is a fine-loamy (547 g kg⁻¹ sand, 109 g kg⁻¹ silt, and 343 g kg⁻¹ clay; 0-10 cm soil depth) Typic Hapludox soil (Soil Survey Staff, 2014), with a bulk density of 1.3 ± 0.1 g cm⁻³. The mean annual temperature is 23.2 °C, and the mean annual precipitation is 1450 mm y⁻¹ (29-year average). A field trial was conducted under no-till since October 2016, with soybean [*Glycine max* (L.) Merrill] grown in the summer, followed by maize (*Zea mays* L.) intercropped with Guinea grass (*Megathyrsus maximus* Jacq. cv Tanzânia) as a relay

crop. Lime and gypsum were applied to ameliorate soil acidity (October 2016 and again in October 2017), before soybean crop. Lime and gypsum rates were calculated based on Quaggio and Van Raij, (1997). Lime rate was applied to raise soil base saturation (BS) to 60% in the topsoil and gypsum rate was calculated by multiplying the clay content in the 20-40 cm soil layer by 6.0 (Quaggio and van Raij, 1997). Nitrogen fertilizer, as ammonium sulphate, was applied annually to maize intercropped with Guinea grass at 0 and 160 kg ha⁻¹ (30 kg N ha⁻¹ at sowing and 130 kg N ha⁻¹ top-dressed, to the respective treatments when maize plants were at the V4-V5 stage).

The experimental design was a 3 × 2 factorial in complete randomized blocks with four replications. The treatments were (i) a control without application of correctives, (ii) 2.9 Mg ha⁻¹ of lime, and (iii) 2.9 Mg ha⁻¹ of lime + 2.1 Mg ha⁻¹ of gypsum, each of these treatments received or not 160 kg ha⁻¹ of N as ammonium sulphate.

3.2.2 Sampling procedure and soil analysis

Three replicate soil samples (0-10 cm) were collected randomly and combined into one composite sample per plot from the field experiment, in August 2018, using a 5.5 cm i.d. stainless steel corer. The samples were passed freely through a 5 mm mesh to remove any discernible roots and stones, ensuring soil homogeneity. Prior to analysis, the soil water content was measured gravimetrically (24 h at 105 °C), for determination of the water holding capacity (WHC). Then, samples were pre-incubated at 60% of WHC and kept stored at 4 ± 1 °C, and rewetted when necessary. Soil carbonate CO₃²⁻ content was assessed with a FOGL bench-top soil calcimeter (BD Inventions P.C., Thessaloniki, Greece), to validate that there was no CO₃²⁻ remaining in the soil from lime application.

Soil pH and electrical conductivity (EC) were determined in 1:5 w/v soil:distilled water suspension using standard electrodes. A sub-sample was air-dried and ground using a ball-mill for total C and N determination by dry combustion with a CHN-2000 analyser (LECO Corp., St. Joseph, MI, USA). Soil solution was extracted with 0.5 M K₂SO₄ (1:5 w/v), from 5 g soil samples following Jones and Willett (2006). The solutions were subsequently stored for analysis at 4 °C in polypropylene vials (Jones and Willet, 2006). To determine the soluble C microbial biomass (MBC), and N microbial biomass (MBN), the soil was first fumigated with chloroform (Vance et al., 1987) for 24 h in the dark at 20 °C. Then, after fumigation, the samples were extracted

with 0.5 M K₂SO₄ (1:5 w/v) as explained before for the non-fumigated samples. Dissolved organic C (DOC) and total dissolved N (TDN) in soil solution were determined using a Multi N/C 3100® analyzer (Analytik Jena, Jena, Germany). Then, MBC and MBN were estimated using value of 0.45 for the constants k_{EC} and k_{EN}, following the methodology used by Kemmitt et al (2006). Ammonium and nitrate were colorimetrically determined from non-fumigated solution, following the procedures of Mulvaney (1996) and Miranda et al. (2001) respectively. Dissolved organic nitrogen (DON) was obtained as the difference between TDN and dissolved inorganic N.

3.2.3 Soil microbial community analysis

Microbial phospholipid fatty acids (PLFA) analysis was carried out to investigate shifts in microbial community structure as described in Bartelt-Ryser et al. (2005). A sub-sample of 25 g of soil from each plot was lyophilized and freeze-stored at -80°C until analysis. Individual PLFA concentrations were determined by GC-MS and taxonomic groups ascribed using the Sherlock® PLFA Method and Tools Package (PLFAD1) by Microbial ID Inc. (Newark, DE, USA). The results for each individual fatty acid that had a concentration higher than 0.5% of the total PLFAs are expressed as a percentage of the total amount of fatty acids (mol% ± SEM) found in a given sample. The biomarkers included: Gram⁺ and Gram⁻ bacteria, actinomycetes, putative arbuscular mycorrhizal fungi (AM Fungi), eukaryote and fungi (Sánchez-Rodríguez et al., 2018). The total PLFAs was used as a proxy of soil microbial biomass. The standard nomenclature chosen was described from Frostegård et al. (1993). Ratios from the individual PLFAs were calculated from different microbial abundance, including: Fungi:Bacteria as an indicator of the nutrients availability; Gram⁺:Gram⁻ as an indicator of soil environmental conditions (e.g. pH, temperature, aeration); ratios used to identify possible stress: mono/polyunsaturated fatty acids (Mono:Poly); and precursor/cyclopropane fatty acids (Gram⁻ : Stress; precursor/ cyclopropane fatty acids; and 16ω/17 cyclo and 18ω/19 cyclo) (Frostegård et al., 1993; Knivett and Cullen, 1965; Sánchez-Rodríguez et al., 2018; Willers et al., 2015).

3.2.4 Total DNA extraction from the soil and 16S sequencing

Total DNA from soil samples was extracted using a PowerSoil DNA isolation kit (MoBio, Carlsbad, EUA), according to the manufacturer's instructions. After extraction, the DNA content was quantified by Qbit methodology. Metabarcoding Libraries of 16S rRNA amplicons corresponding to V3–V4 variable region were prepared by single PCR with double-indexed fusion primers as described by Fadrosh et al. (2014). The 16S rRNA annealing part of forward primer corresponded to modified 515F (5'-GTGCCAGCMGCCGCGGTAA-3'); and reverse primer corresponded to modified 806R (5'-GGACTACHVGGGTWTCTAAT3') (Merkel et al., 2015). Single-step PCR amplification was performed by qPCRMix-HS™ SYBR mastermix (Evrogen, Russia) using the following conditions: 30 cycles of denaturation at 95°C for 15 s; primer annealing at 58°C, 15 s; DNA elongation at 72°C, 25 s, followed by final DNA elongation for 5 min at 72°C. Purification of PCR products was done using the QIAEX II Gel Extraction Kit (Qiagen, Germany). The quality of the final libraries was assessed using the electrophoresis in agarose gel (Korzhenkov et al., 2019).

Libraries were sequenced with MiSeq™ Personal Sequencing System technology of Illumina Inc. (San Diego, CA, USA) using paired-end 250-bp reads (Korzhenkov et al., 2019). Demultiplexing was performed as described previously (Fadrosh et al., 2014). All reads were subjected to stringent quality filtering, using fastx toolkit and tagcleaner to remove barcodes and adapters from reads. After demultiplexing, OTU picking and taxonomy assignment was made using QIIME 1.9.1 in open-reference mode and Silva132 database, with all parameters set to default values (Korzhenkov et al., 2019).

3.2.5 Statistical analysis

All statistical procedures were performed in R (version 3.6.3, The R Foundation for Statistical Computing, Boston, MA, USA) and RStudio (version 1.2.5019, RStudio, Vienna, Austria). The normality and homoscedastic for the data of the experiments were analysed by Shapiro-Wilk and Bartlett tests. Non-normally distributed data were log₁₀-transformed prior to analysis. Factorial ANOVA based on a completely randomized block design with two factors (soil correctives, fertilizer) was carried out for the soil characteristics properties and PLFA analysis. Tukey's HSD *post-hoc* test

was used to identify treatment differences. Statistical significance is reported at the 5% level of significance. Finally, Pearson correlations were carried out between soil properties and PLFA markers.

In addition to the taxonomy assignment for DNA, normalization method was applied to relative abundance of 16S rRNA gene V3-V4 amplicon reads selected groups. The groups were selected according to their relation to soil pH and N cycle, following Zhalnina et al. (2015). Since the presence of these groups is not in huge percentage, we took an average of the relative abundance among replicates on each type of culture. These averages were normalized among the maximum number got for each of these taxonomic groups, getting values between 0 and 1.

3.3 RESULTS

3.3.1 *Soil characteristics*

Soil pH was increased by at least 1.0 unit in treatments with L and L+G compared with control, while N fertilizer application decreased soil pH by at least 1.0 unit, regardless of soil amendments (Table 1). In addition, higher EC was obtained for N fertilized treatments than those non-fertilized. No differences were observed for soil organic C (OC) or C:N ratio in the soil. However, total N content increased ~7% with N application and was higher for L+G treatment when compared with sole L application. Moreover, NO_3^- -N increased with N fertilizer, but no difference was observed for L+G, and only fertilized treatments had differences, with lower values for L+G treatment compared with control. In contrast to NO_3^- -N, there was a difference regarding NH_4^+ -N among soil correctives in non-fertilized plots, where lime applied alone had the lowest NH_4^+ -N content. In addition, N application increased NH_4^+ -N for L applied alone, decreased its content for L+G, or had no influence in the control.

Dissolved organic C (DOC) was affected by the soil correctives only in the absence of N fertilizer, and was 32% greater for L+G than for lime alone (Table 2). Similar to DOC, dissolved organic nitrogen (DON) was altered only in the absence of N fertilizer, and the L+G treatments had 65% more DON than lime applied alone. Furthermore, DOC and DON contents were higher for L+G in unfertilized treatments than those that received N. The DOC:DON ratio was higher in limed treatments in comparison with control, with no effect of N application, except for L+G without N

fertilizer that was similar to control and lower than treatments with lime and L+G under N addition. The microbial biomass C (MBC) was 23% lower for lime applied alone than the control and L+G, irrespective of N management, and 23% lower for unfertilized treatments than those fertilized, irrespective of soil correctives. In contrast to MBC, microbial biomass N (MBN) was 23% higher for unfertilized relative to fertilized treatments, with no difference for soil correctives.

Table 1 - Values of pH and electric conductivity (EC), Organic C, total N, C:N ratio, and inorganic N in the soil as affected by base correction (lime $\pm$ gypsum) and fertilizer management, ($\pm$ N). Values represent mean $\pm$ SEM ($n = 4$)

Corrective	Fertilizer	pH	EC	Organic C	Total N	C:N	NO ₃ ⁻ -N	NH ₄ ⁺ -N
			$\mu\text{S cm}^{-1}$	g kg^{-1}	g kg^{-1}	Ratio	mg kg^{-1}	mg kg^{-1}
Control	No Fertilizer	5.1 $\pm$ 0.2bA	61 $\pm$ 5B	12.2 $\pm$ 0.6	1.3 $\pm$ 0.1abB	9.3 $\pm$ 0.1	23.6 $\pm$ 3.3B	0.9 $\pm$ 0.1a
	Fertilized	4.4 $\pm$ 0.2bB	173 $\pm$ 21A	12.3 $\pm$ 0.9	1.4 $\pm$ 0.1abA	8.7 $\pm$ 0.2	51.2 $\pm$ 6.2aA	1.0 $\pm$ 0.1
Lime	No Fertilizer	6.2 $\pm$ 0.3aA	52 $\pm$ 6B	11.1 $\pm$ 0.5	1.2 $\pm$ 0.1bB	9.7 $\pm$ 0.7	23.4 $\pm$ 0.7B	0.5 $\pm$ 0.1bB
	Fertilized	5.4 $\pm$ 0.3aB	171 $\pm$ 19A	12.5 $\pm$ 0.8	1.4 $\pm$ 0.0bA	9.0 $\pm$ 0.4	39.6 $\pm$ 3.8abA	0.9 $\pm$ 0.0A
L+G	No Fertilizer	6.5 $\pm$ 0.1aA	89 $\pm$ 4B	13.7 $\pm$ 0.7	1.4 $\pm$ 0.1aB	10.0 $\pm$ 0.4	28.5 $\pm$ 3.3	1.2 $\pm$ 0.0aA
	Fertilized	5.7 $\pm$ 0.3aB	150 $\pm$ 10A	14.0 $\pm$ 1.1	1.5 $\pm$ 0.1aA	10.0 $\pm$ 0.5	34.6 $\pm$ 1.1b	0.8 $\pm$ 0.2B
<i>P</i> value								
C		<0.001	0.748	0.052	0.029	0.247	0.216	0.005
F		<0.001	<0.001	0.357	0.005	0.112	<0.001	0.981
C $\times$ F		0.981	0.042	0.697	0.256	0.878	0.034	0.002

Means followed by a common letter within a column are not significantly different by the Tukey-test at the 5% level of significance. Lowercase letters differ in between correctives; Uppercase letter differ in between fertilizer.

Table 2 - Dissolved organic pool and microbial-derived C and N in the soil as affected by base correction (lime $\pm$ gypsum) and fertilizer management, ($\pm$ N). Values represent mean $\pm$ SEM ($n = 4$).

Corrective	Fertilizer	DOC	DON	DOC:DON	MBC	MBN
		mg kg ⁻¹	mg kg ⁻¹	Ratio	mg kg ⁻¹	mg kg ⁻¹
Control	No Fertilizer	194 $\pm$ 8bA	5.3 $\pm$ 1.1ab	32 $\pm$ 2b	121 $\pm$ 5aB	14.3 $\pm$ 0.9A
	Fertilized	156 $\pm$ 7B	7.8 $\pm$ 2.7	20 $\pm$ 4b	160 $\pm$ 7aA	11.3 $\pm$ 0.9B
Lime	No Fertilizer	158 $\pm$ 4c	2.7 $\pm$ 0.6b	49 $\pm$ 4a	102 $\pm$ 13bA	14.4 $\pm$ 1.1A
	Fertilized	174 $\pm$ 6	4.8 $\pm$ 1.3	52 $\pm$ 5a	90 $\pm$ 13bB	9.5 $\pm$ 1.2B
L+G	No Fertilizer	231 $\pm$ 15aA	7.7 $\pm$ 0.6aA	31 $\pm$ 4bB	77 $\pm$ 21aB	15.8 $\pm$ 1.6A
	Fertilized	141 $\pm$ 14B	2.4 $\pm$ 0.6B	49 $\pm$ 6aA	144 $\pm$ 19aA	13.5 $\pm$ 1.8B
<i>P value</i>						
C		0.131	0.231	<0.001	0.024	0.173
F		<0.001	0.19	0.454	0.021	0.009
C $\times$ F		<0.001	0.004	0.011	0.051	0.638

Means followed by a common letter within a column are not significantly different by the Tukey-test at the 5% level of significance. Lowercase letters differ in between correctives; Uppercase letter differ in between fertilizer.

3.3.2 Soil microbial community analysis

The total phospholipid fat acid (PLFA) concentration in soil did not differ for soil correctives nor fertilizer management, averaging 58.5 nmol g⁻¹ (Table 3). However, abundance of the different taxonomic groups was affected by certain treatments. Soil correctives reduced Gram+ bacteria from 40% in control to 39% (on average) for L and L+G treatments, while it increased the Gram- bacteria from 34% in control to 36% (on average) for the treatments with L and L+G. However, the *Actinomyces* percentage decreased by 1.2% for L+G, compared to control and L lime applied alone. Similar to the Gram-, eukaryote increased for L and L+G treatments compared with the control. Conversely, Gram+ and Gram- bacteria, *Actinomyces* and eukaryote percentage were not affected by N management. The highest percentage of arbuscular mycorrhizal fungi (AM Fungi) was observed for L+G treatment without N, followed by lime applied alone. Nitrogen application reduced de AM Fungi when L or L+G were applied, in 0.99% and 1.63%, respectively. However, non-mycorrhizal fungi (Fungi) were not affected by soil correctives nor N fertilizer.

The shifts in microbial structures (PLFA; Table 3) were also reflected on changes in PLFA ratios (Table 4). The Fungi:Bacteria biomass ratio increased with L+G application compared to control, in unfertilized treatments. Moreover, Gram+:Gram- ratio decreased with soil correctives, while Gram-:Stress ratio increased compared with the control. Other indexes of soil stress, mono:polyunsaturated fatty acids (Mono:Poly) and Gram-:Stress ratio also increased for lime and L+G treatments. However, the Gram-:Stress ratio was lower in treatments with the co-addition of L+G and N fertilizer, suggesting increased stress over Gram- community by N fertilizer.

Our analyses revealed that soil pH and MBN were the characteristics most strongly correlated with PLFA taxonomic groups and ratios (Figure 1). Soil pH had strong to moderate positive correlation with AM Fungi (0.88), Eucaryote (0.54), PLFA (0.53) Gram- bacteria (0.50), Fungi:Bacteria ratio (0.79); Gram-:Stress ratio (0.81), Mono:Poly ratio (0.68); in addition to negative correlation with Gram+ bacteria (-0.71), Gram+:Gram- ratio (-0.66), *Actynomicetes* (-0.65). A positive correlation was found between soil MBN and AM Fungi (0.60), PLFA (0.51) and Fungi:Bacteria ratio (0.61), and a negative correlation was observed with *Actynomicetes* (-0.60) and Gram+:Gram- ratio (-0.43).

Table 3 - Factorial analysis of the total amount of PLFAs and taxonomic groups in the soil as affected by base correction (lime $\pm$ gypsum) and fertilizer management, ($\pm$ N). Values represent mean $\pm$ SEM ($n = 4$).

Corrective	Fertilizer	PLFA	Gram+	Gram-	Actinomycetes	AM Fungi	Eukaryote	Fungi
		nmol g ⁻¹	%	%	%	%	%	%
Control	No Fertilizer	58.4 $\pm$ 0.7	40.2 $\pm$ 0.5a	34.1 $\pm$ 0.8b	18.1 $\pm$ 0.2a	3.47 $\pm$ 0.13 c	1.22 $\pm$ 0.13b	2.58 $\pm$ 0.11
	Fertilized	53.4 $\pm$ 4.0	40.7 $\pm$ 0.6a	34.2 $\pm$ 0.9b	18.2 $\pm$ 0.4a	3.05 $\pm$ 0.08	1.13 $\pm$ 0.10b	2.39 $\pm$ 0.28
Lime	No Fertilizer	54.0 $\pm$ 3.6	39.1 $\pm$ 0.7b	34.9 $\pm$ 0.7a	17.7 $\pm$ 0.4a	4.35 $\pm$ 0.18 bA	1.44 $\pm$ 0.14a	2.50 $\pm$ 0.17
	Fertilized	58.7 $\pm$ 4.5	38.5 $\pm$ 0.2b	36.2 $\pm$ 0.2a	17.8 $\pm$ 0.3a	3.36 $\pm$ 0.08 B	1.52 $\pm$ 0.08a	2.34 $\pm$ 0.11
L+G	No Fertilizer	65.1 $\pm$ 4.4	38.6 $\pm$ 0.3b	35.6 $\pm$ 0.5a	16.1 $\pm$ 0.5b	5.13 $\pm$ 0.23 aA	1.73 $\pm$ 0.11a	2.45 $\pm$ 0.03
	Fertilized	61.8 $\pm$ 3.0	39.0 $\pm$ 0.7b	35.7 $\pm$ 0.4a	17.4 $\pm$ 0.3b	3.50 $\pm$ 0.04 B	1.64 $\pm$ 0.09a	2.61 $\pm$ 0.19
<i>P</i> value								
C		0.0889	<0.001	0.002	0.004	<0.001	0.002	0.791
F		0.6785	0.768	0.116	0.098	<0.001	0.116	0.627
C $\times$ F		0.3744	0.206	0.245	0.227	0.004	0.245	0.476

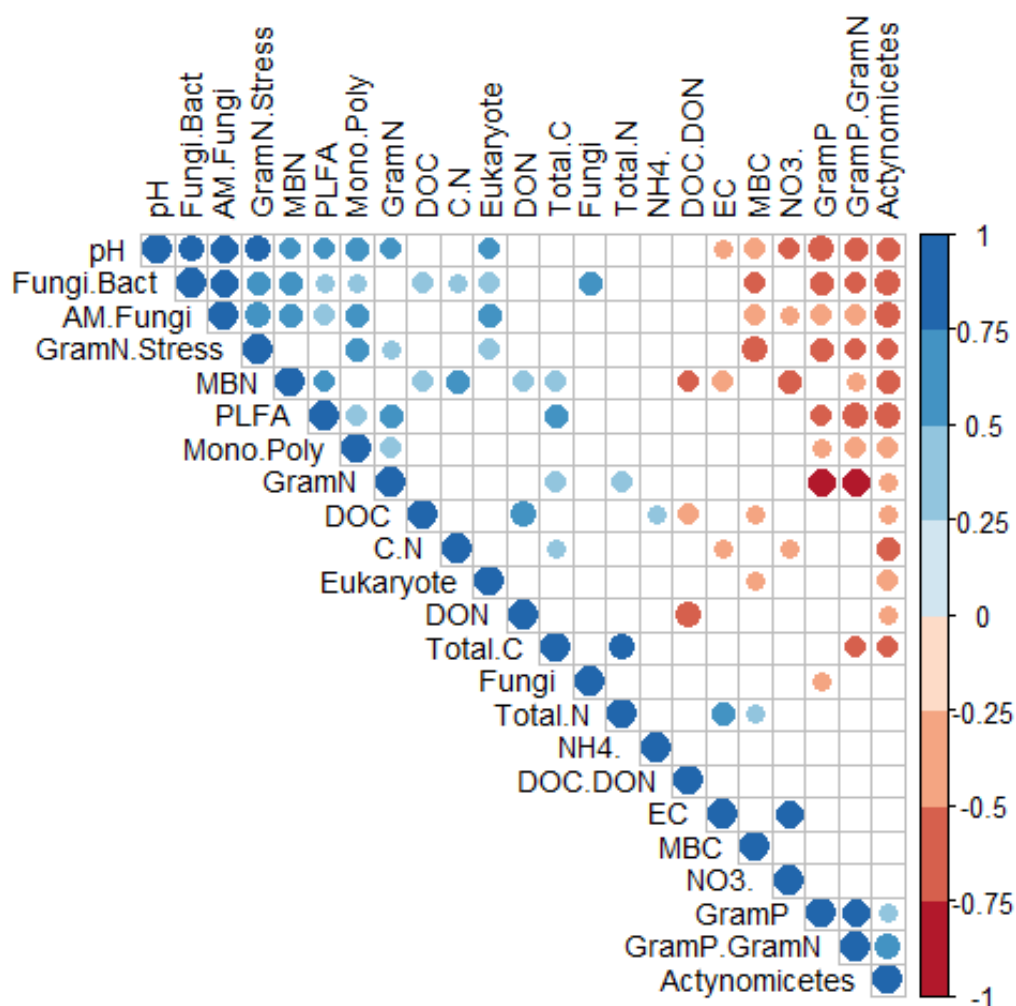
PLFA = phospholipid fatty acid; AM Fungi = arbuscular mycorrhiza. Means followed by a common letter within a column are not significantly different by the Tukey-test at the 5% level of significance. Lowercase letters differ in between correctives; Uppercase letter differ in between fertilizer.

Table 4 - Factorial analysis of the ratios based on PLFAs in the soil as affected by base correction (lime $\pm$ gypsum) and fertilizer management, ($\pm$ N). Values represent mean $\pm$ SEM ($n = 4$)

Corrective	Fertilizer	Fungi : Bact	Gram+ : Gram-	Mono : Poly	Gram- : Stress
Control	No Fertilizer	0.082 $\pm$ 0.003b	1.52 $\pm$ 0.04a	6.78 $\pm$ 0.39b	0.51 $\pm$ 0.04b
	Fertilized	0.073 $\pm$ 0.003	1.53 $\pm$ 0.07a	6.02 $\pm$ 0.42b	0.49 $\pm$ 0.03b
Lime	No Fertilizer	0.093 $\pm$ 0.004ab	1.46 $\pm$ 0.06b	7.29 $\pm$ 0.46a	0.86 $\pm$ 0.06a
	Fertilized	0.081 $\pm$ 0.003	1.39 $\pm$ 0.01b	7.35 $\pm$ 0.38a	0.76 $\pm$ 0.05a
L+G	No Fertilizer	0.109 $\pm$ 0.007aA	1.36 $\pm$ 0.02b	7.61 $\pm$ 0.21a	0.90 $\pm$ 0.02aA
	Fertilized	0.078 $\pm$ 0.004B	1.40 $\pm$ 0.03b	6.91 $\pm$ 0.30a	0.61 $\pm$ 0.07abB
<i>P</i> value					
C		0.008	0.003	0.037	<0.001
F		<0.001	0.988	0.133	0.002
C $\times$ F		0.042	0.286	0.467	0.035

PLFA = phospholipid fatty acid; AM Fungi = arbuscular mycorrhiza. Means followed by a common letter within a column are not significantly different by the Tukey-test at the 5% level of significance. Lowercase letters differ in between correctives; Uppercase letter differ in between fertilizer.

Figure 1 - Pearson correlation of PLFA groups, PLFA ratios and soil properties (significance at $p \leq 0.05$). Different sizes of circles and colour mean different correlation, showed on the scale. PLFA = PLFA concentration; AM.Fungi = arbuscular mycorrhiza fungi; GramN = Gram negative bacteria; Eukaryote = Eukaryote; Fungi = Fungi; GramP = Gram positive bacteria; Actinomycetes = Actinomycetes; Fungi.Bact = Fungi : Bacteria ratio; GramP.GramN = Gram Positive : Gram Negative ratio; Mono.Poly= Mono : Poly ratio; GramN.stress = Gram Negative : Stress ratio; pH = soil pH; EC = Electrical conductivity; Total.C = Total carbon; Total.N = Total nitrogen; C.N = C:N ratio; DOC = Dissolved organic C; DON = dissolved organic N; DOC.DON = DOC : DON ratio MBC = Microbial biomass C; MBN = Microbial biomass N; NO₃. = Nitrate (NO₃⁻-N); NH₄. = ammonium (NH₄⁺-N)



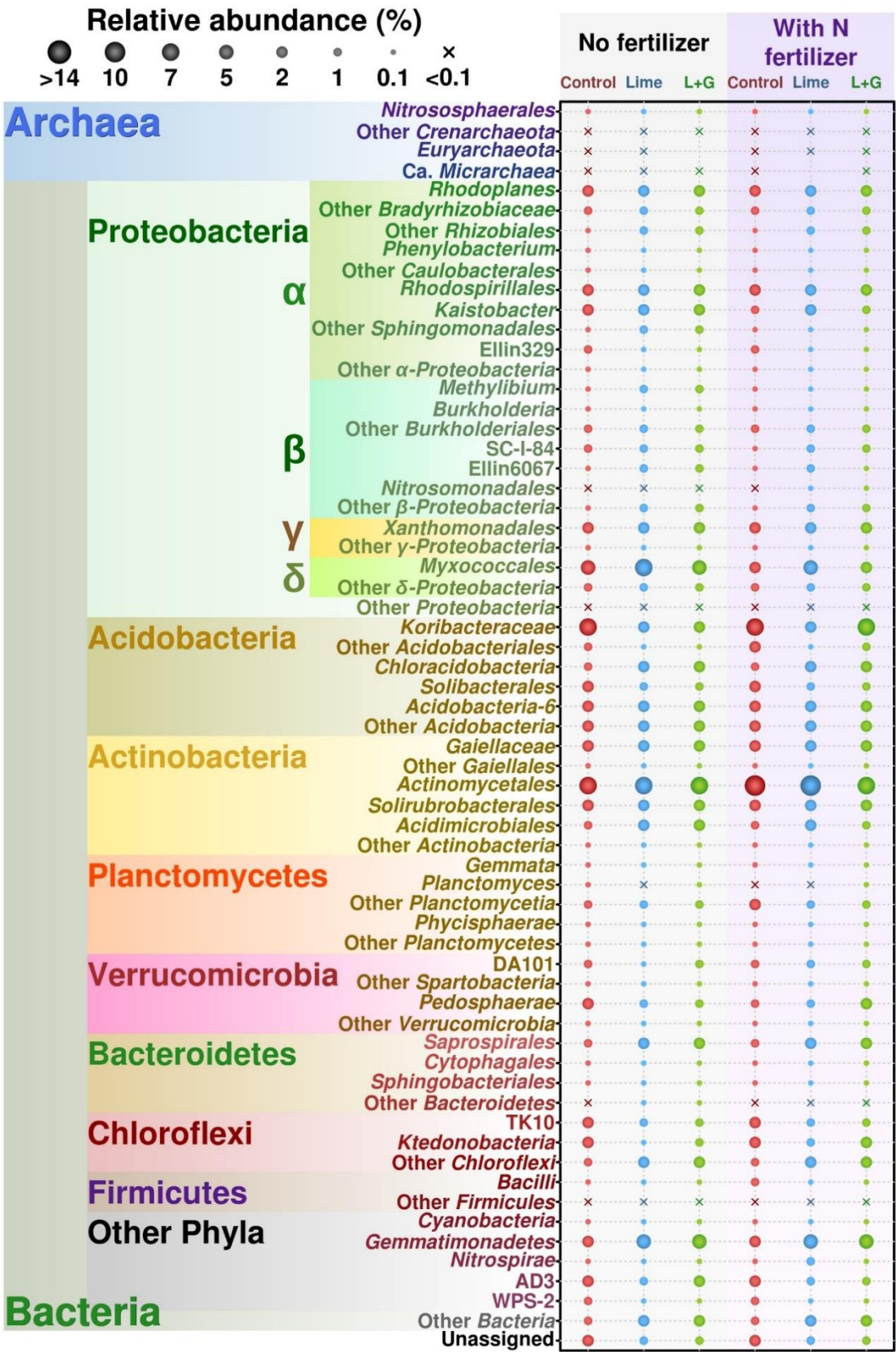
SOURCE: the author.

3.3.3 DNA 16S sequencing

A total of 2,300 operational taxonomic unit (OTU) were identified in our soil samples. From those, ~1% was related to the Archaea Domain and ~97% Bacteria, while Unassigned genes represented ~2%. As related to the Archaea domain, the greater phylum was *Crenarchaeota*, with 1% composed by the genus of *Nitrososphaerales* and <0.1% of each other genus (*Crenarchaeota* spp, *Euryarchaeota* spp, Ca. *Micrarchaea* spp; Figure 2). Overall Bacteria presented relative abundance of 57 different genera of bacteria. In general, the greatest phylum in Bacteria was *Proteobacteria* (21 genera; 30%), followed by *Actinobacteria* (6 genera; 19%) and *Acidobacteria* (6 genera; 19%). Together the above phyla encompassed more than two thirds of total bacterial genera (69%). Other abundant bacterial genera belonged to the phyla: *Planctomycetes* (3.2%), *Verrucomicrobia* (4.0%), *Bacteroidetes* (3.1%), *Chloroflexi* (6.4%) and *Firmicutes* (0.7%).

The class *Alphaproteobacteria* (α - *Proteobacteria*; 14%), from *Proteobacteria* phylum was composed by the family *Bradyrhizobiaceae*, where the genus *Rhodoplanes* represented 5% of total bacteria genera, while Other *Bradyrhizobiaceae* and Other *Rhizobiales* consisted in 2% each. The genera *Rhodospirillales* and *Kaistobacter*, in *Alphaproteobacteria*, also composed 5% of total 16RNAs each. The β -*proteobacteria* class (β -*Proteobacteria*; 6.2%) was composed by the genera *Methylibium*, *Burkholderia*, Other *Burkholderiales*, SC-I-84 and *Ellin6067*, that variety from 1.0% to 2% of relative abundance. The *Nitrosomonadales* genus consisted in <0.1% except for the L and L+G treatments with N addition, that had 1.0% each. The *Gammaproteobacteria* class (γ - *Proteobacteria*; 3.7%), was composed by 2.9% of relative abundance of the genus *Xanthomonadales* and 0.8% of other *Gammaproteobacteria* genera. *Deltaproteobacteria* class (δ - *Proteobacteria*; 6.8%), *Myxococcales* genus had higher relative abundance for L and L+G treatments, with 7.2% and 6.8%, against 5.3% in unfertilized control. Similarly, L and L+G totalled 5.3% and 4.9%, respectively, against 2.8% in control, when N fertilizer was applied. While other *Deltaproteobacteria* did not differ and composed 2% of relative abundance.

Figure 2 - Relative abundance (%) of 16S rRNA gene V3-V4 amplicon reads as affected by soil amendment (lime ± gypsum) and fertilizer management (± N).

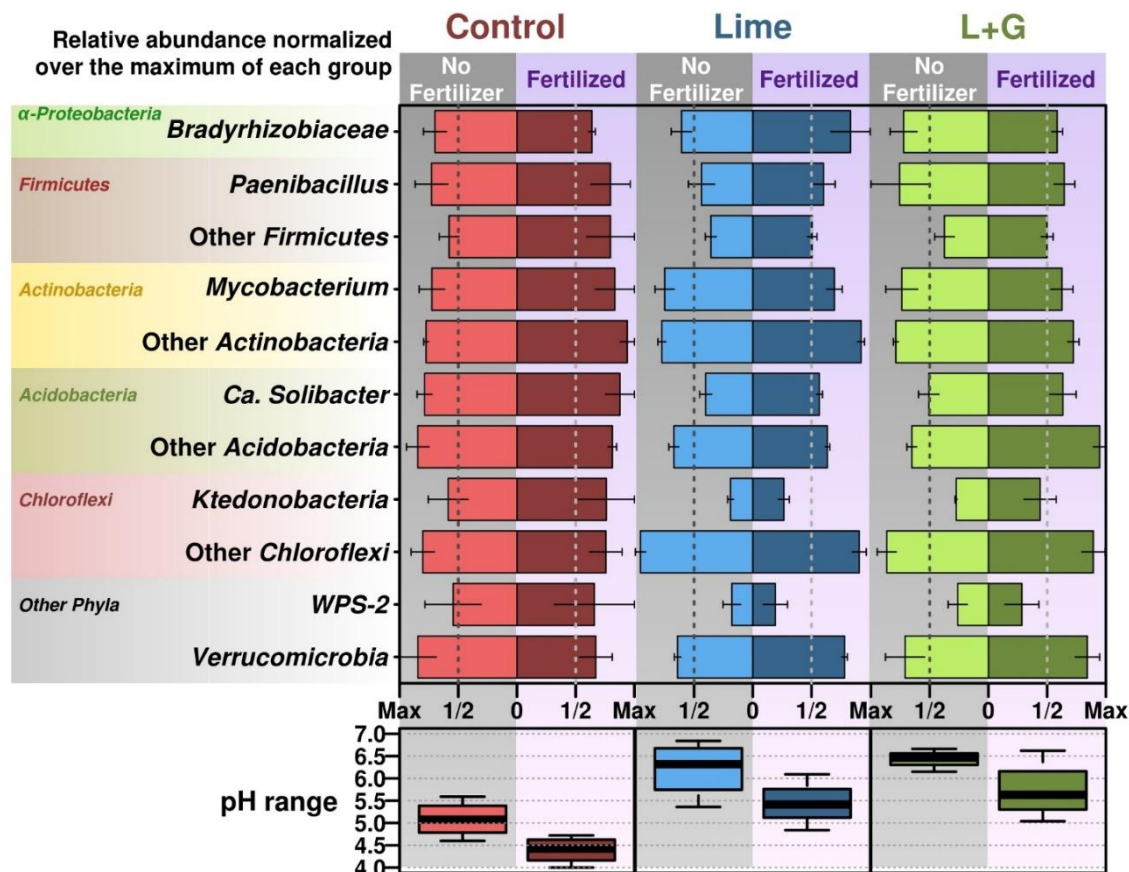


SOURCE: the author.

The relative abundance normalized over the maximum of each group was accessed to compare the effect of treatments related to soil pH change on bacterial phyla (Figure 3). The *Proteobacteria* phylum presented differences for the *Bradyrhizobiaceae* family (*Alphaproteobacteria* class), that presented relative abundance closest to the max for L treatment with the addition of N fertilizer, while control and L+G fertilized treatments presented around half of relative abundance. The *Paenibacillus* genus from *Firmicutes* phylum, presented half or less than half of relative abundance for L treatment fertilized or not with N, respectively. While control and L+G presented more than half relative abundance of *Paenibacillus* genus, irrespective of fertilizer management. However, Other *Firmicutes* groups, from *Firmicutes* phylum presented similar trend for treatments with L and L+G with less than half of relative abundance for those treatments without N-fertilizer, and half relative abundance for the fertilized L or L+G.

The *Mycobacterium* genus from *Actinobacteria* phylum was affected by the co-addition of N-fertilizer, L and L+G, showing lower relative abundance (close to half), compared with other treatments close to maximum relative abundance. In addition, other *Actinobacteria* groups in the phylum only decreased the relative abundance for L+G with N-fertilizer. The relative abundance of the *Ca. Solibacter* genus, from the *Acidobacteria* phylum, decreased with L and L+G application, showing half or less than half relative abundance for limed treatments. However, the relative abundance of Other *Acidobacteria* groups also decreased, but no more than half of relative abundance. In addition, L+G treatments fertilized showed relative abundance of Other *Acidobacteria* groups close to the maximum, similar with control treatments. The relative abundance of *Ktedonobacteria* class, from the *Chloroflexi* phylum, decreased for treatments with L and L+G, irrespective of N management, with relative abundance close to zero for limed treatments. Conversely, Other *Chloroflexi* groups in the phylum had relative abundance close to the maximum. The *WPS-2* phylum relative abundance also decreased for close to zero relative abundance for L and L+G treatments, irrespective of N management. In addition, the general relative abundance of *Verrucomicrobia* phylum was greater than half, but presented lower relative abundance for non fertilized treatments with L and L+G, or for the control under N addition.

Figure 3 - Relative abundance of 16S rRNA gene V3-V4 amplicon reads normalized over the maximum of each group related with changes in soil pH as affected by soil amendment (lime $\pm$ gypsum) and fertilizer management ($\pm$ N).



SOURCE: the author.

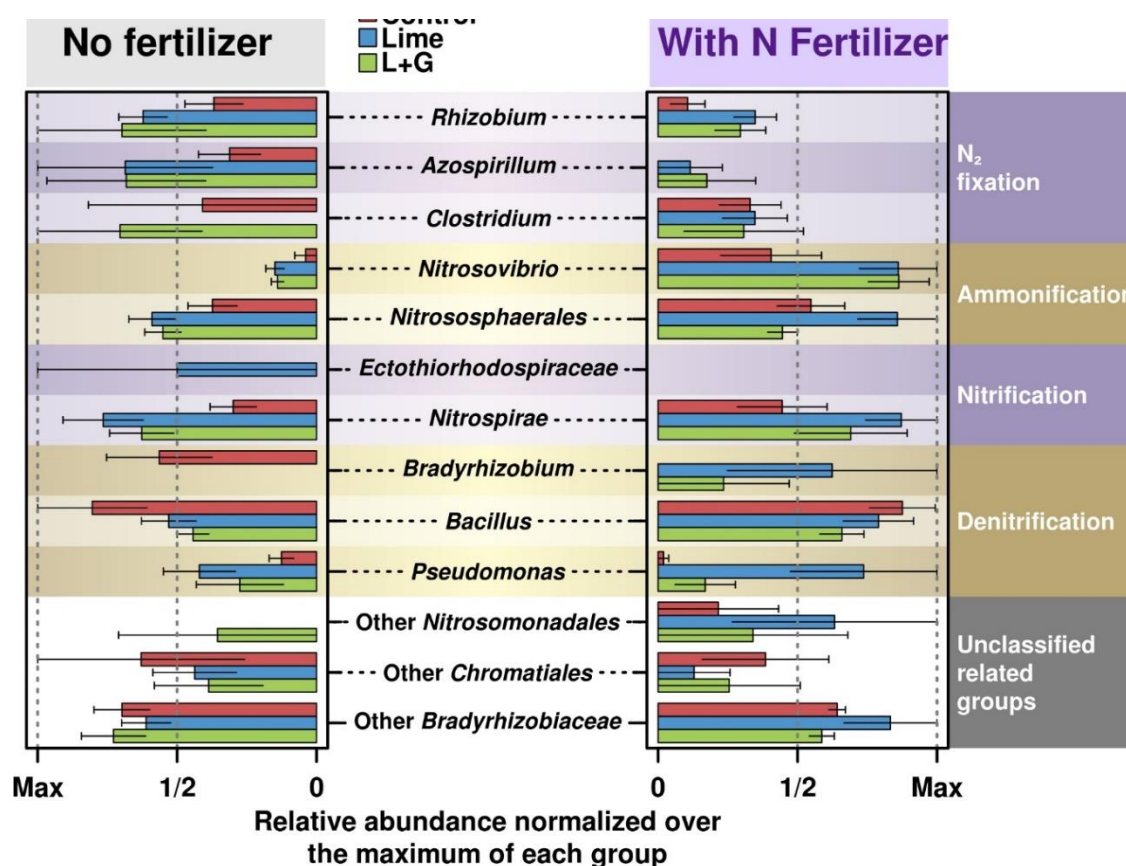
The normalized relative abundance of groups related with N cycle (i.e., groups participating on N_2 fixation, ammonification, nitrification and denitrification process) can be seen in Figure 4. The genera related with N_2 fixation affected by treatments were *Rhizobium*, *Azospirillum* and *Clostridium*. The relative abundance of these three genera decreased to less than half with N fertilizer application. The relative abundance in *Rhizobium* and *Azospirillum* genera for L and L+G treatments, and in *Clostridium* genus for L+G treatment, was greater for the treatments without N fertilizer in comparison with control.

The genera *Nitrosovibrio* and *Nitrososphaerales*, related with the ammonification process, were affected by N fertilizer and soil amendments application (Figure 4). The relative abundance of *Nitrosovibrio* genus was close to zero for treatments without N, and increased with N fertilizer application, showing greater relative abundance (close to the maximum) with the co-addition of L and L+G. The

relative abundance of *Nitrososphaerales* genus was close to half for treatments without N, with highest abundance for L and L+G. However, the relative abundance of *Nitrososphaerales* genus increased for limed treatment coupled to N addition.

SOURCE: the author.

Figure 4 - Relative abundance of 16S rRNA gene V3-V4 amplicon reads normalized over the maximum of each genera related with nitrogen cycle as affected by soil amendment (lime $\pm$ gypsum) and fertilizer management ($\pm$ N).



SOURCE: the author.

The genera related with nitrification and affected by treatments were *Ectothiorhodospiraceae* and *Nitrospirae* (Figure 4). The *Ectothiorhodospiraceae* genus only appear for limed treatment without N fertilizer. The relative abundance of *Nitrospirae* genus was higher for L, followed by L+G, and control, showing increasing relative abundance with N fertilizer, but similar behaviour when comparing soil correctives.

Genera related with denitrification as affected by treatments were *Bradyrhizobium*, *Bacillus* and *Pseudomonas* (Figure 4). The relative abundance of the

Bradyrhizobium genus was negligible for L and L+G without fertilizer, only appearing for fertilized treatments. When comparing control treatments, the relative abundance of the *Bradyrhizobium* genus was higher than half without N, and negligible for control with N fertilizer application. The relative abundance of the *Bacillus* genus presented similar pattern among soil correctives, with higher relative abundance in control, followed by L and then L+G, but with higher general relative abundance in fertilized treatments, compared with those unfertilized. The relative abundance of the *Pseudomonas* genus was greater for the co-addition of L and N-fertilizer compared to other treatments that have presented less the half of relative abundance.

Unclassified related groups belong to the *Protobacteria* phylum, being the Other *Nitrosomonadales* (order of the *Betaprotobacteria* class); the other *Chromatiales* (order of the *Gammaproteobacteria* class); and the other *Bradyrhizobiaceae* (a family of the *Alphaproteobacteria* class). The Other *Nitrosomonadales* relative abundance was greater for the L treatment with N addition. The relative abundance of Other *Chromatiales* were lower than half for treatments with L and L+G, irrespective of N fertilizer management, and higher than half in control without N. The relative abundance of other *Bradyrhizobiaceae* was increased by L treatment coupled to N-fertilizer.

3.4 DISCUSSION

Influence of lime and gypsum amendment and N fertilizer on soil properties

Liming is a common agricultural recommendation to alleviate soil acidity (Holland et al., 2018; Ritchey et al., 1980). The effects of surface application of lime increasing soil pH in the short-term observed here are in line with Inagaki et al. (2017) and Garbuio et al. (2011). Although ammoniacal fertilization reduce soil pH in comparison with unfertilized treatments, it did not change the effectiveness of surface liming, similar to the results obtained by Garbuio et al. (2011). In addition, N fertilizer addition increased the soil EC, irrespective of soil correctives. Furthermore, despite the expected increase in total N content due to N-fertilizer addition, L+G treatment also had higher N content than L sole. Unlike to the results presented here, Inagak et al (2017) and Garbuio et al., (2011) observed minimal effects of lime and gypsum on the total N in short-term. However, the OC content and the C:N ratio were not affected by soil correctives and N management. A weak correlations between soil pH and soil C

and N stocks was also found in previous studies (Kemmitt et al., 2006). However, benefits from liming and N fertilizer increasing in C and N stocks in the long-term could be obtained, such as the increase in deposition of organic materials due to the tight stoichiometric balancing of C and N in SOM (Carmeis Filho et al., 2017; Fornara et al., 2011; Inagaki et al., 2017). In addition, the increase in soil NO_3^- -N and NH_4^+ -N content as affected by liming and N-fertilizer addition are in accordance with other reports (Gibbons et al., 2014; Kemmitt et al., 2006; Rosolem et al., 2017).

Generally, higher soil pH is associated with higher microbial activity, and with positive effects on MBC and MBN, although effects of N addition on MBC and MBN can be controversial (Aye et al., 2018; Kemmitt et al., 2006). We found that N-fertilizer distinctly affected MBC and MBN concentrations, increasing MBC and decreasing MBN. Opposite results were reported by Aye et al. (2018), who found a positive effect of N addition on MBN, but a suppressive effect on MBC. Further, liming applied alone led to lower MBC, similar to Wachendorf (2015), who observed a decrease in MBC following lime addition. In addition, although the application of gypsum does not affect soil pH, the co-addition with lime benefits soil fertility and microbial activity, affecting N and C mineralization in soil (Carmeis Filho et al., 2017; Pivetta et al., 2019). As observed by Pivetta et al. (2019), the L+G treatment increased the DOC content in soil solution. Soil labile organic fractions of C and N, such as DOC, DON, MBC and MBN comprise the most active fractions of soil organic matter, (Shahid et al., 2017; Sun et al., 2020). In addition to provide short-term C and N resources for microorganisms and plants, the soil labile organic fractions of C and N respond sensitively to various management regimes (Shahid et al., 2017; Sun et al., 2020).

Microbial soil community and structure

The total PLFA concentration found here (58.5 nmol g⁻¹ dry soil in average), is in line with previous results under similar conditions (ranging from 26-89 nmol g⁻¹ dry soil; Ai et al., 2015; Börjesson et al., 2012). However, it was higher in comparison with PLFA concentration observed by Sarto et al. (2020) in Cerrado and pasture soils in Brazil (32 and 29 nmol g⁻¹ dry soil, respectively). Many studies have suggested that the main drivers of microbial diversity in soils are soil pH and N inputs (Abalos et al., 2020; Rousk et al., 2011; Zhalnina et al., 2015). Microbial biomass, estimated by total PLFA, is reported to be negatively correlated to soil pH (Sarto et al., 2020; Zhang et al., 2016),

and to decrease with the addition of ammonium-based fertilizer (Rousk et al., 2011; Zhalnina et al., 2015). Furthermore, general changes in the microbial structure due to increased soil pH were associated with higher microbial biomass (Abalos et al., 2020). However, Rousk et al. (2011) only observed changes in total PLFA, and their biomarkers for soil pH below 4.5. In contrast, despite the present soil pH have ranged from 4.4 to 6.5 (Table 8), the total PLFA concentration did not change (Table 12).

Although soil correctives and N fertilizer application did not affect total PLFA in soil, they affected different taxonomic groups. A shift in the Gram+:Gram- ratio may indicate stressful conditions such as low oxygen, suboptimal pH or water content, or low nutrient supply (Frostegård et al., 2011; Kaneda, 1991; Veum et al., 2019). A decrease in the Gram+:Gram- ratio with increasing soil pH was reported by Fornara et al. (2011) and Abalos et al. (2020). In the present study, the decrease in the Gram+:Gram- ratio derived from the decrease in Gram+ bacteria and increase in Gram- bacteria. Conversely, Abalos et al. (2020), only observed an increase in Gram- bacteria due to liming. Gram+ bacteria are assumed to possess specific mechanisms which enable them to survive in acidic environments (Cotter and Hill, 2003), being more relatively stable and resistant to stress (Sánchez-Rodríguez et al., 2018; Zelles, 1999). The stress related ratios, Mono:Poly and Gram-:Stress, also increased with L and L+G treatments, showing the potential of liming in mitigate soil microbial pH related stress (Abalos et al., 2020).

In addition, liming leads to an increased Fungi:Bacteria ratio (Abalos et al., 2020; Rousk et al., 2010). Environmental processes such as nutrient cycling, organic matter decomposition and C sequestration have been associated with fluctuations in the Fungi:Bacteria ratio (Willers et al., 2015), where a higher Fungi:Bacteria biomass ratio is linked to increased C storage potential in soils (Malik et al., 2018). In fact, the Fungi:Bacteria ratio had positive correlations with DOC and C:N ratio. Similar correlation involving fungal biomass and C:N ratio was reported by Veum et al. (2019). Generally, increasing nutrient availability is suggested by Ngosong et al., (2010) to support the fungal growth. However, AM Fungi community is sensitive to N fertilizer addition (Rousk et al., 2011). In addition, AM Fungi abundance was increased by both corrective treatments, in line with Abalos et al. (2020) and Rousk et al., (2011). Furthermore, saprophytic fungi was not affected by treatments, leading to strong correlation between AM Fungi with Fungi:Bacteria ratio. The Fungi:Bacteria ratio was lower than 0.11, being in accordance with the literature that ranges from 0.02 to 0.35

(Abalos et al., 2020; Ngosong et al., 2010; Rousk et al., 2011). Generally, arable soils comprise low saprotrophic fungi biomass as compared with bacteria (Joergensen and Wichern, 2008; Ngosong et al., 2010). Furthermore, Bacteria are suggested to be more sensitive than fungi to soil environment changes caused by agricultural practices (Dong et al., 2017; Li et al., 2020).

The metagenomic analysis of the soils revealed relative abundance of 57 different microbial genera, being in accordance with the findings presented by Bossolani et al. (2021), who performed a field experiment with similar agricultural system, and close to our field trial. The relative abundances of the phylum *Proteobacteria* (~30%) and *Acidobacteria* (~18%), were similar to those observed by Silveira et al. (2021). The dominance of *Proteobacteria* and *Acidobacteria* has been demonstrated in recent descriptions of bacterial communities in Cerrado soils, with some variations in the abundance of these two groups depending on plant composition, soil characteristics, or rainfall seasonality (Babujia et al., 2016; Silveira et al., 2021; Silveira Sartori Silva et al., 2019; Souza et al., 2016). However, Silveira et al. (2021) and Bossolani et al. (2021), evaluating a long-term effect of liming observed a higher abundance of *Chloroflexi* (14% and 9%, respectively) than observed here (6%).

Soil pH has been shown to be a good predictor of bacterial phylum and genus level abundance in soils (Silveira et al., 2021; Wan et al., 2020; Zhalnina et al., 2015). The differences obtained here were found at family and genus level. The *Actinobacteria* phylum is known for its metabolic versatility to be oligotrophic, with high resistance to adverse conditions, thus showing greater relative abundance under low pH (e.g control and N fertilized treatments). Our findings are in accordance with previous results where negative correlation between *Actinomycetes* and soil pH was found (Bossolani et al., 2021; Silveira et al., 2021). The *Bradyrhizobiaceae* family (*Alphaproteobacteria* class) and *Bradyrhizobium* relative abundances were negatively affected by L and L+G, agreeing with other researchers (Bossolani et al., 2020; Silveira et al., 2021). Despite the annual inoculation of soybean seeds with *Bradyrhizobium* strains (Babujia et al., 2016; Hungria and Mendes, 2015), Brazilian soils are considerate free of *Bradyrhizobium* compatible with soybean (Souza et al., 2016). In addition, *Bradyrhizobium* are related to the denitrification metabolism, and classified as oligotrophic organisms, (Souza et al., 2016; Zhalnina et al., 2015), thus being favoured by lower pH and N-fertilizer addition.

The *Paenibacillus* genus from *Firmicutes* phylum, is found to be positive correlated with soil pH (Zhalnina et al., 2015). However, the relative abundance of *Paenibacillus* was negatively affected by N fertilizer addition, as observed by Li et al., (2020). The relative abundance of other *Firmicutes* groups, however, were favoured by N fertilizer addition. The *Firmicutes* phylum includes the classes *Bacilli* and *Clostridia*, known as spore-forming microorganisms, providing high resistance of these bacteria to unfavourable environments (de Araujo et al., 2017; Souza et al., 2016). The genera *Bacillus* and *Paenibacillus* from the *Firmicutes* phylum, in addition to *Pseudomonas* and *Azospirillum* from *Proteobacteria* phylum, are representatives of plant growth-promoting bacteria (de Araujo et al., 2017; Lammel et al., 2018). In addition, both *Bacillus* and *Pseudomonas* are related with denitrification metabolism (Merloti et al., 2019), showing higher relative abundance under N addition. Although showing different response to shifts in the soil pH and corrective management, the genera *Rhizobium*, *Azospirillum* and *Clostridium*, which are related with N₂ fixation, have the relative abundance decreased with N-fertilizer addition (Kuhner et al., 2000; Lammel et al., 2018; Zhalnina et al., 2015). Conversely, the genera involved in ammonification metabolism (*Nitrosovibrio* and *Nitrososphaerales*), and nitrification metabolism (*Ectothiorhodospiraceae* and *Nitrospirae*) have higher relative abundance with N fertilizer addition (Cassman et al., 2019; Liu et al., 2021).

3.5 CONCLUSION

Our findings suggest that, even in short term, the surface application of lime and gypsum are modulating soil microbial community and favouring the relative abundance of beneficial microorganisms. Increases in Fungi:Bacteria ratio, as observed with the addition of both soil correctives, are frequently associated to a more efficient use of C. In addition the decrease in Gram+:Gram- ratio, by increasing the Gram- relative abundance suggests that in addition to alleviate soil acidity, soil correctives are increasing plant-derived C sources, and will probably benefit microbial biomass. Yet, increasing the AM Fungi relative abundance could reflect in soil nutrient cycle and plant nutrient acquisition. Moreover, despite the fact that the application of lime, gypsum and nitrogen did not cause changes in Bacteria domain at the phylum-level in short-term, it has demonstrated benefits in the relative abundance N cycle bacterial genera. Thus, these earliest findings indicate that even short-term changes in soil pH is a critical

driver of shifts in the microbial community composition, and the acidity alleviation is beneficial to C and N cycles.

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FINAL CONSIDERATIONS

Soil acidity is one of the most important limitations to crop production in the world. Although the primary aim of liming being overcoming soil acidity, it impacts the chemical, biological and physical properties of soils, thereby affecting microbial activity, transformations of C and N in soils and GHG fluxes. Despite the well-known benefits of lime application on crop production, there are some drawbacks and negative impacts of liming in the net climate change. As the CO₂ emissions produced during neutralization of acidity, or possible effect on nitrate leaching due to stimulation of lime in mineralization and nitrification process have been discussed. During the incubation test, treatments with lime and gypsum have resulted in greater ¹⁴CO₂ respired, irrespective of N fertilizer management or soil depth. However, the CUE showed no difference between treatments, in accordance with the accumulated CO₂-C emissions observed under field condition. In addition, the CUE of ¹⁴C-glucose was similar between soil layers, demonstrating potential for C sequestration. Throughout the experiment it became clear that the use of strategies such as application of gypsum and nitrogen fertilizer, as well as the system of cultivation with legumes in combination with grasses, favoured the cycling of N and increased the fixation of C. The cultivation of soybean in fact reduced the dependence on N fertilizer in the system, while intercropping maize and guinea grass favoured N cycle, since compared with the literature it was observed general low NO₃⁻-N leaching and great plant biomass production. In addition, despite the partial negative N balance, the application of lime promoted an increase of C and N in the systems, in addition to greater grain production. It was demonstrated that the results of CUE and the mineralization of C and N are not directly related to the change in pH caused by the application of lime. The microbial community profile revealed that the abundance of beneficial microbial groups, such as AM fungi and N cycle bacteria, as favoured by alleviating soil acidity stress with the addition of lime and gypsum. Among the changes obtained by the soil amendments, increasing in Fungi:Bacteria ratio, and decreasing in Gram+:Gram- ratios are suggested to be a microbial community with better CUE. Thus, these earliest findings indicate that even in short-term changes in soil pH is a critical driver of change for the microbial community composition, and the alleviation on acidity is beneficial to C and N cycles.

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