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Topsoil and subsoil C and N turnover are affected by superficial lime and gypsum application in the short–term

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Abstract

Alleviation of subsoil acidity with lime or gypsum increases carbon (C) accumulation in deep layers by stimulating root growth and C and nitrogen (N) inputs at depth. However, the effects of these amendments combined with N fertilization on soil CO_2 emissions remain controversial. We evaluated the effects of superficial lime and gypsum application and N–fertilizer on C and N dynamics and microbial C use efficiency (Mic_{CUE}) in samples taken from the topsoil (0–10 cm) and subsoil (40–60 cm) of a no–till field experiment carried out in Brazil. We performed short–term laboratory incubation with ^{14}C–glucose and ^{14}C–arginine to assess C and N mineralization dynamics. Liming increased topsoil pH but had no effect on subsoil acidity. Higher content of organic C, total N, and microbial biomass C and N were found in the topsoil. The addition of soil corrective and N fertilizer had no effect on Mic_{CUE} of added ^{14}C–glucose. However, the Mic_{CUE} of ^{14}C–arginine was affected~~only influenced~~ by the soil layer, and~~which~~ was highe~~rst~~ in the subsoil. After the addition of arginine, net NH_4^+–N production was highest in the topsoil control, while net NO_3^-–N content was highest with~~in the treatment~~ lime + gypsum plus~~with~~ residual N in the same layer. We can conclude that while lime and gypsum ~~may~~ ameliorate soil acidity, they have minimal effect on the C cycling t~~hrough~~through the microbial biomass, particularly in the subsoil.

Key words: soil acidity, ammonium sulphate, ^{14}C–glucose, ^{14}C–arginine, carbon use efficiency

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 (CO_2), methane (CH_4), and nitrous oxide (N_2O), known to affect global warming (Pachauri et al., 2014). Soil respiration is largely controlled by the activity of microbial communities, which are typically limited by the availability of labile C and N forms (de Sosa et al., 2018; Glanville et al., 2016). While C and N turnover rates decrease with increasing soil depth, the addition of C substrates can readily stimulate the microbial biomass in the subsoil, therefore suggesting a C–limited lifestyle (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 Al^{3+} in the subsoil, which inhibits root growth (Aye et al., 2018).

Liming is highly effective at alleviating soil acidity, but due to its chemical dissolution, lime is considered a net source of CO_2 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

63 microbial biomass is frequently restricted to the top 5 cm in tropical soil under no–till (Ferrari
64 Neto et al., 2021). Although gypsum generally does not affect soil pH, its higher solubility
65 compared with lime promotes the movement of SO_4^{2-} through the soil profile carrying Ca^{2+}
66 which helps alleviate Al^{3+} activity, promoting subsoil fertility and rooting (Ritchey et al.,
67 1980; Vieira Fontoura et al., 2019). The low solubility and movement of lime in the soil
68 profile have led to the recommendation of gypsum ($CaSO_4.H_2O$) as a soil conditioner
69 alongside lime (Ritchey et al., 1980), especially for no–till systems (de Vargas et al., 2019;
70 Vieira Fontoura et al., 2019). Therefore, a combination of lime and gypsum may promote
71 subsoil fertility and increase root growth deeper into the soil profile (Pivetta et al., 2019;
72 Vieira Fontoura et al., 2019), increasing C inputs into the subsoil (Inagaki et al., 2017; Jones
73 et al., 2018b). Thus, liming associated with gypsum is expected to have a positive effect on
74 the size of the microbial biomass and its metabolic activity (Garbuio et al., 2011; Inagaki et
75 al., 2017; Kemmitt et al., 2006), with subsequent effects on C mineralization and N
76 mineralization throughout the soil profile (Garbuio et al., 2011; Wachendorf, 2015).
77 In N–limited systems, such as deep soil horizons, microorganisms are more C
78 inefficient since excess C is directly respired as CO_2 instead of incorporating it into the
79 microbial biomass through anabolic processes (Sinsabaugh et al., 2013). Thus, the adoption
80 of practices to increase soil C reserves deeper in the soil profile where O_2 concentration is
81 lower may represent an important strategy for long–term C accumulation, helping to mitigate
82 climate change (Lorenz and Lal, 2018). Considering that lime and gypsum combined with N–
83 fertilizer application may improve subsoil activity and remove N limitation for the microbial
84 community, we hypothesized that surface application of lime and gypsum would alleviate
85 soil acid stress (from Al^{3+}/H^+) and promote greater microbial C use efficiency (Mic_{CUE}) in
86 both the topsoil and subsoil. Further, we hypothesized that the co–addition of lime and
87 gypsum would enhance microbial subsoil responses in comparison to lime alone.

2. Material and methods

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^{-1} 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 sulphate 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. 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 (CO_3^{2-}) content was measured with a FOGL bench–top soil calcimeter (BD Inventions P.C., Thessaloniki, Greece) to confirm that no lime–derived CO_3^{2-} remained in the soil. Soluble C and N were extracted from moist soil (5 g) with 25 ml of 0.5 M K_2SO_4 (200 rev min^{-1}, 30 min), followed by storage of the extracts at 4 °C (Jones and Willet, 2006).

2.3. Soil characterization

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, MBC and MBN were determined, while dissolved organic C (DOC) and total dissolved N (TDN) were determined in the unfumigated

137 samples. Fumigated and unfumigated samples were extracted in a 0.5 M K_2SO_4 solution (1:5
138 soil–to–K_2SO_4 solution; w/v), and determined using a Multi N/C 3100® analyzer (Analytik
139 Jena, Jena, Germany). MBC and MBN were calculated using a extraction efficiency value
140 (k_{EC}, k_{EN}) of 0.45 (Kemmitt et al., 2006). Ammonium and nitrate in the K_2SO_4 extracts were
141 determined colorimetrically following the salicylic acid procedure of Mulvaney (2018) and
142 vanadate method of Miranda et al. (2001), respectively. Dissolved organic N (DON) was
143 calculated as the difference between TDN and dissolved inorganic N (NH_4^+–N + NO_3^-–N).
144 Sub–samples were air–dried, ground using a ball–mill, and total C and N (TN) determined
145 using a CHN–2000 analyzer (Leco Corp., St. Joseph, MI, USA). Since no carbonate–C was
146 found in soil, we assumed that all the C is present in organic forms and therefore designated
147 this major pool as organic C (OC).

148

149 *2.4. Microbial glucose mineralization and substrate carbon use efficiency*

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

158 For each sample, 5 g of soil moist was placed into sterile 50 cm^3 polypropylene
159 containers. Subsequently, 0.25 ml of ^{14}C–labeled D–glucose (Sigma–Aldrich Ltd., Poole,
160 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 $^{14}CO_2$. 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 $^{14}CO_2$ 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 $^{14}CO_2$ determination. The remaining K_2SO_4 extract was used to determine the NH_4^+–N and NO_3^-–N content in the soil, as described earlier. Microbial immobilization of ^{14}C–glucose ($^{14}C_{immob}$) at the end of the incubation was estimated as follows:

$$^{14}C_{immob} = {}^{14}C_{total} - {}^{14}C_{K_2SO_4} - {}^{14}CO_2 \quad \text{(Eqn. 1)}$$

where $^{14}C_{total}$ is the total amount of ^{14}C–glucose added to the soil at time–zero, $^{14}C_{K_2SO_4}$ is the amount of ^{14}C recovered in the 0.5 M K_2SO_4 extract, and $^{14}CO_2$ is the total amount of ^{14}C recovered as $^{14}CO_2$ 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 v.12.5 (Systat Software Ltd., London, UK):

$$^{14}C_{soil} = (Mic_{catab} \times \exp^{-k_c \times t}) + (Mic_{anab} \times \exp^{-k_a \times t}) \quad \text{(Eqn. 2)}$$

where $^{14}C_{soil}$ is the amount of ^{14}C remaining in the soil over time (t), Mic_{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 Mic_{catab} and Mic_{anab} pools, respectively. The first pool (Mic_{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, Mic_{CUE} can be calculated as follows (Jones et al., 2018c):

$$Mic_{CUE} = Mic_{anab} / (Mic_{catab} + Mic_{anab}) \quad \text{(Eqn. 3)}$$

The half–life for the first mineralizable pool ($Mic_{catab}\ t_{½}$) can be calculated from:

$$Mic_{catab}\ t_{½} = ln(2) / k_c \quad \text{(Eqn. 4)}$$

2.5. Microbial arginine mineralization and substrate carbon 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 moist 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 $^{14}CO_2$, 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 $^{14}CO_2$ 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 $^{14}CO_2$ determination. Microbial immobilization of the arginine–derived ^{14}C ($^{14}C_{immob}$) 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):

$$Mic_{CUE} = {}^{14}C_{immob} / ({}^{14}C_{immob} + {}^{14}CO_2) \quad \text{(Eqn. 5)}$$

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. Net NH_4^+–N and NO_3^-–N production of arginine–treated soils were calculated over the incubation period relative to zero–time samples of each treatment. In addition, the $^{14}CO_2$:NH_4^+–N ratio was obtained by the division of the total $^{14}CO_2$ respired (expressed in mg $^{14}CO_2$ kg^{-1} of soil) by the net NH_4^+–N production (expressed in mg N kg^{-1} of soil) after 48 h from arginine addition.

2.6. Statistical analysis

All statistical procedures were performed in R (version 3.6.3, The R Foundation for Statistical Computing, Boston, MA, USA) with RStudio (v1.2.5019, RStudio, Vienna, Austria) as front–end. The normality and homoscedasticity of the data were analyzed by Shapiro–Wilk and Bartllet tests, respectively. 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 × fertilizer × 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.

3. Results

3.1. Soil characteristics

The pH results showed~~had an~~ interaction of~~between~~ the corrective with the~~and~~ soil layer, as well as of~~between~~ N fertilizer with the~~and~~ soil layer (Table 1). In particular, pH in the amended treatments was ~1.2 unit greater than the control in~~for~~ the topsoil ~~only~~ (Fig 1A). Further, soil pH ~~control treatment~~ was similar in ~~both~~ the topsoil and subsoil in the control treatment, and was also similar in the subsoil of~~to those~~ treatments ~~that~~ receiv~~inged~~ lim~~e~~ 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 showed~~, with~~ the lowest values, with~~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 μS 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).

Organic C was affected interactively by~~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 × 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).

A three–way interaction was observed for i~~I~~norganic 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 in the subsoil content compared with all topsoil treatments, regardless of the N management, ~~(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 compared ~~in comparison~~ with lime + gypsum without N in the same layer and all treatments in the subsoil, with exception of the~~to~~ control with N (Fig. 2D). Microbial biomass–N was affected by~~presented the main effect of~~ the correctives (Table 1), where lime + gypsum was 15% higher, on average, compared with lime alone and the unamended control (Fig. 2E). In addition, a fertilizer × layer interaction was also found (Table 1), where MBN content was higher without N compared with~~relative to~~ residual N addition in the topsoil, while the subsoil showed~~had~~ the lowest values and was not affected by N management (Fig. 2F).

3.2. Microbial glucose mineralization and carbon use efficiency

The main effect of corrective was observed for $^{14}CO_2$ 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 $^{14}C_{K2SO4}$ in the deep layer (Fig. 3B). The $^{14}C_{immob}$ was influenced by the main effect of corrective (Table 1) similarly to the $^{14}CO_2$ 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 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.*, Mic_{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, Mic_{catab} $t_{½}$ was influenced by a three–way interaction (Table 1). In this regard, the unamended control without N addition in the subsoil had a lower Mic_{catab} $t_{½}$ 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).

3.3. Microbial arginine mineralization and carbon 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 × fertilizer × layer interaction (Table 1). Overall, 32% more $^{14}C_{K2SO4}$ 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 $^{14}C_{immob}$ and Mic_{CUE} were influenced by the main effect of layer, similar to $^{14}CO_2$ (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 $^{14}CO_2$:NH_4^+–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 (Table1), where lime + gypsum with N in the subsoil had a higher ratio compared~~in comparison~~ with the other~~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 × fertilizer and corrective × 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 ≈ 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).

4. Discussion

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 withtout 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

358 the lime + gypsum treatment compared with lime applied alone, they did not differ relative to
359 the control. As observed here, the application of lime ± gypsum has previously been shown to
360 have minimal effect on TN concentration, at least in the short–term, showing a similar trend
361 as the corrective × layer interaction for OC (Garbuio et al., 2011; Inagaki et al., 2017).
362 However, an increase in TN stocks could be obtained over longer timescales, with the
363 increase in deposition of organic materials due to the tight stoichiometric balancing of C and
364 N in SOM (Carmeis Filho et al., 2017). In addition, we suggest that the highest TN
365 concentration with residual N fertilization can be explained by a higher biomass return to the
366 soil (Diekow et al., 2005).

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

383 arginine assay, and the rapid microbial removal of labile DON from solution as suggested by
384 Jones et al. (2018b).

385 Similar to OC and TN concentrations, MBC and MBN were higher in the topsoil
386 compared with the subsoil (Jones et al., 2018b). In addition, a positive effect of N fertilization
387 on MBC was observed in the subsoil for the control treatment. Aye et al. (2018) reported a
388 positive effect of N addition on MBN, but the effect on MBC was suppressive, while
389 Wachendorf (2015) observed an increase in both MBC and MBN with N application to soil
390 without root litter. Liming and high soil pH also generally have positive effects on MBC and
391 MBN, with a subsequent higher microbial activity (Aye et al., 2018; Kemmitt et al., 2006).
392 However, under residual N, lime had lower MBC compared with control treatment in the
393 topsoil, while similar MBN was obtained in control and lime applied alone (main effect of
394 corrective). These findings are in line with Wachendorf (2015), who observed a decrease in
395 MBC following lime addition. Liming can induce a short–term increase in the available C
396 substrate, often increasing microbial activity, but microbial biomass may not change or even
397 decrease due to the progressive depletion of these substrates (Badalucco et al., 1992;
398 Wachendorf, 2015).

399

400 *4.2. Carbon partitioning and microbial carbon use efficiency in response to glucose*

401 The addition of a low concentration of ^{14}C–glucose allowed us to mimic natural rates
402 of C turnover in the soil (de Sosa et al., 2018; Glanville et al., 2016; Jones et al., 2018b). The
403 $^{14}CO_2$ emission and kinetic parameters from the decay equation (data not shown) did not
404 exhibit any major difference in ^{14}C processing between the topsoil and subsoil following
405 glucose addition, even with a higher MBC and MBN in the topsoil. The activity of soil
406 microbial communities and labile C availability are often considered the main drivers of soil
407 respiration (Glanville et al., 2016). However, a slight difference in the ^{14}C–glucose turnover

(*i.e.*, $^{14}CO_2$ evolved and $^{14}C_{immob}$) 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 μg 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 $^{14}C_{K2SO4}$ 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 Mic_{catab} $t_{½}$ < 0.5 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} $t_{½}$ 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).

4.3. Carbon partitioning and microbial carbon use efficiency in response to arginine

Unlike ^{14}C–glucose, the turnover of ^{14}C–arginine was different between the two layers, with higher $^{14}CO_2$ 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 $^{14}CO_2$ evolution in the first 6 h across soil depths, but mineralization rates were similar after 48 h. Garbuio et al. (2011) observed higher $^{14}CO_2$ 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 μmol 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 $^{14}CO_2$ 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 $^{14}CO_2$ evolution) with increasing soil C:N ratio (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 $^{14}CO_2$ 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}:

458 alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine,
459 phenylalanine, proline, serine, threonine, tyrosine, and valine; final concentration 5 mM)
460 turnover in agricultural soils, Jones et al. (2005) observed no effect on $^{14}CO_2$ evolved
461 following N fertilizer application, while soil pH slightly decreased. The authors concluded
462 that total microbial activity is likely a key determinant of amino acid turnover since
463 management of agricultural soils (*e.g.*, the effect of N, P, and K fertilizer addition; grazing;
464 pH manipulation with lime addition; vegetation cover and shifts comparing grassland versus
465 arable; and drainage) have shown little effect on topsoil. Therefore, the higher MBC and
466 MBN in the topsoil could be related to ^{14}C–arginine turnover response.

467 The Mic_{CUE} for ^{14}C–arginine had an opposite trend from its turnover, with the highest
468 value verified in the subsoil. Kemmitt et al. (2006) reported lower amino acid turnover with
469 deep soil horizons, reflecting the higher microbial activity found in upper layers. Therefore,
470 the higher Mic_{CUE} in the subsoil is likely explained by the lower microbial biomass turnover
471 in this layer, which is supported by the higher amount of ^{14}C–arginine immobilized and much
472 lower $^{14}C_{K2SO4}$ recovered in the topsoil than in the subsoil. However, the $^{14}CO_2$:NH_4^+–N ratio
473 was similar between the topsoil and subsoil. The treatment lime + gypsum with residual N
474 had the lowest efficiency regarding ^{14}C–arginine mineralization, with a higher proportion of
475 $^{14}CO_2$ evolved per molecule of NH_4^+–N mineralized. Evaluating the selective N
476 mineralization relative to C from arginine, Fujii et al. (2020) reported $^{14}CO_2$:NH_4^+–N ratios
477 varying from 1.0 to 1.5 after 96 h incubation, which is similar to our range (0.74–1.78) for a
478 48–h incubation period. Moreover, Fujii et al., 2020 suggest that C and N mineralization was
479 decoupled for treatments with the highest $^{14}CO_2$:NH_4^+–N ratio (1.5), indicating differences in
480 intercellular activities in the arginine degradation into urea (CH_4N_2O) and ornithine
481 ($C_5H_{12}N_2O_2$). Since there was no difference for Mic_{CUE} of ^{14}C–arginine related to corrective
482 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 $^{14}C_{K2SO4}$ 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 NH_4^+–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)

5. Conclusions

The surface application of lime and gypsum only alleviates soil acidity in the topsoil. However, despite a slight increase of $^{14}CO_2$ 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, $^{14}CO_2$

508 evolved from glucose suggests that both topsoil and subsoil microbial biomass are similarly
509 active. On the other hand, the Mic_{CUE} of ^{14}C–arginine is only affected by the soil layer, where
510 the subsoil is higher than the subsoil, while net NH_4^+–N and NO_3^-–N productions are affected
511 by the amendments. However, the data indicates that these results are not directly related to
512 soil pH, the main response to lime and/or gypsum, or with the C:N ratio, as previously
513 described in the literature. The N cycling in both topsoil and subsoil is limited by upstream
514 elements in the N cycle (*i.e.*, substrate availability for protease action). Thus, the influence of
515 lime and gypsum seems to be related to nutrient availability, nitrification, microbial activity,
516 and microbial community structure. Further research is needed to unravel the profile of
517 microbial communities, among other soil properties, caused by the application of lime and
518 gypsum and demystify the theory that these correctives are harmful sources of CO_2 to the
519 atmosphere.
520
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532

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663

664

665 **Table**

666

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

Variable	Factor						
	Corrective (C)	**Fertilizer (F)**	**Layer (L)**	**C×F**	**C×L**	**F×L**	**C×F×L**
Soil property							
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–glucose assay							
$^{14}CO_2$	**0.008**	0.198	0.948	0.144	0.819	0.371	0.431
$^{14}C_{K2SO4}$	**0.002**	0.099	**<0.001**	0.320	**0.009**	0.340	0.244
$^{14}C_{immob}$	**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} $t_{½}$	**<0.001**	0.275	**<0.001**	**<0.001**	**0.012**	0.081	**0.003**
^{14}C–arginine assay							
$^{14}CO_2$	0.089	0.426	**<0.001**	0.161	0.168	0.052	0.066
$^{14}C_{K2SO4}$	**<0.001**	**0.003**	**<0.001**	**0.012**	**0.002**	**0.001**	**0.016**
$^{14}C_{immob}$	**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
$^{14}CO_2$:NH_4^+–N	**<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**

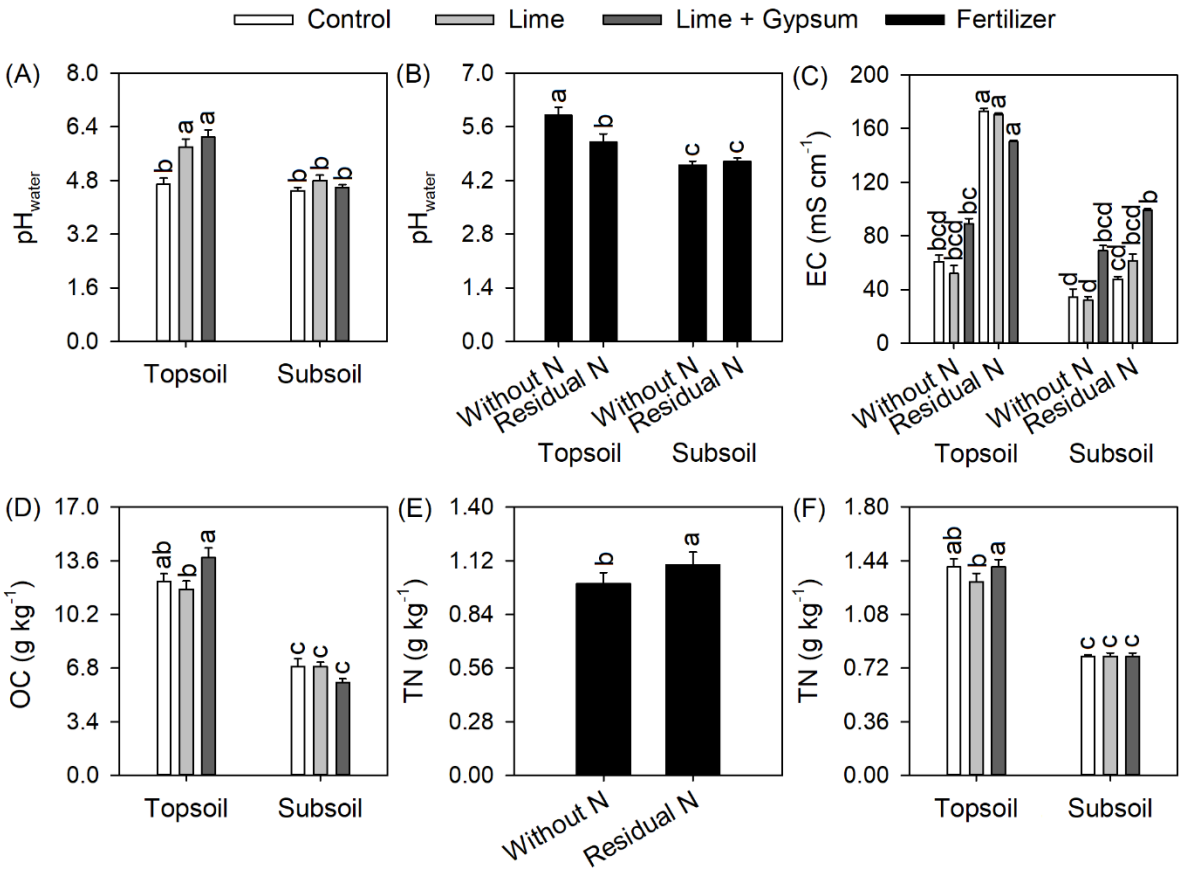
671 pH: pH in water; EC: electrical conductivity; OC: organic C; TN: total N: IN: inorganic N; DOC: dissolved organic C;
672 DON: dissolved organic N; MBC: microbial biomass C; MBN: microbial biomass N; $^{14}CO_2$: $^{14}CO_2$ evolved from the soil;
673 $^{14}C_{K2SO4}$: ^{14}C recovered in the soil solution; $^{14}C_{immob}$: ^{14}C immobilized into the microbial biomass; Mic_{CUE}: microbial C use
674 efficiency of the substrate; Mic_{catab} $t_{½}$: half–life of the first mineralizable pool; $^{14}CO_2$:NH_4^+–N: the ratio between $^{14}CO_2$

675 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
676 incubation; Net NO_3^-–N: net NO_3^-–N production at the end of the incubation.
677

678

679 **Figure captions**

680

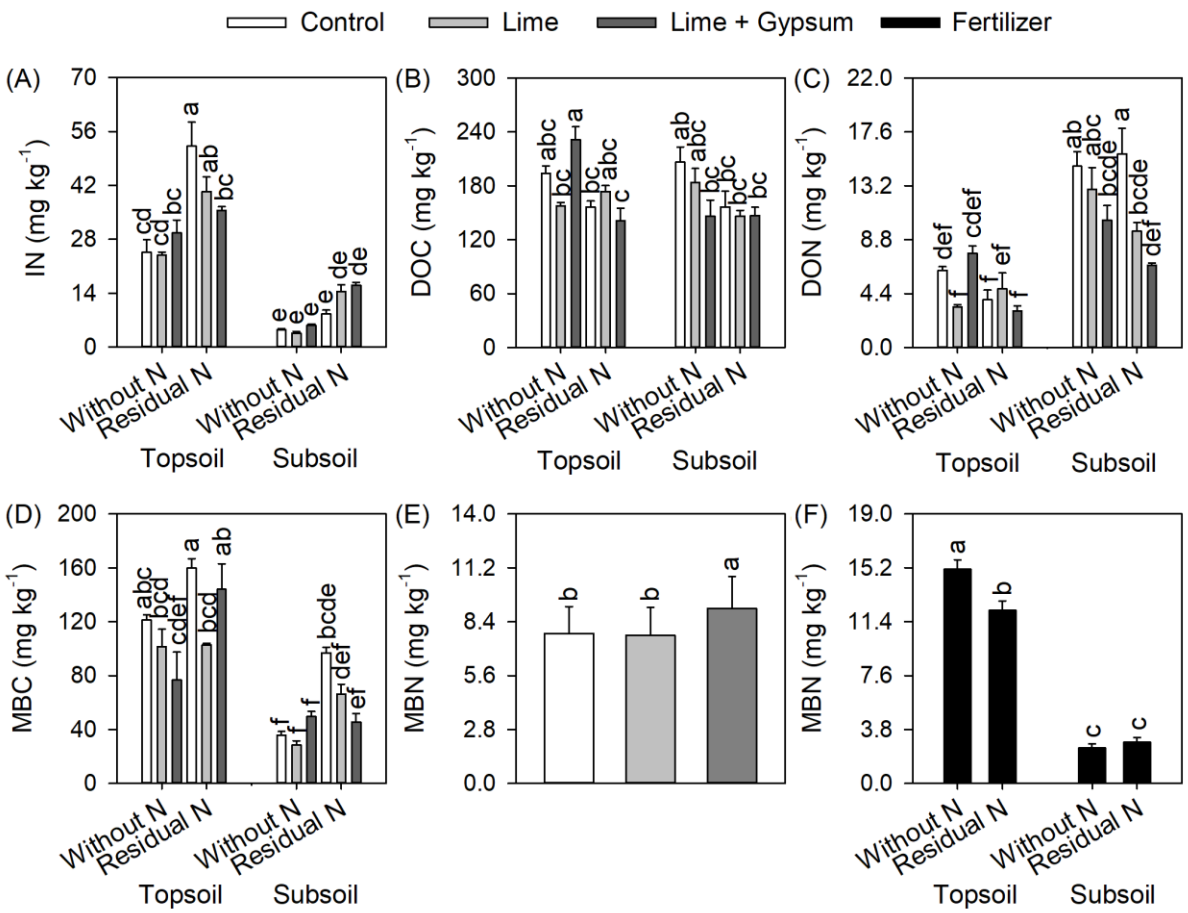


681

682 **Fig. 1.** Chemical soil properties measured before the incubation assays. pH affected by
683 corrective × layer and fertilizer × layer interactions (A and B, respectively); electrical
684 conductivity (EC) affected by a corrective × fertilizer × layer interaction (C); organic C (OC)
685 affected by a corrective × layer interaction (D); total N (TN) affected by the main effect of
686 fertilizer and a corrective × layer interaction (E and F, respectively). Only significant effects
687 (Table 1) were shown. The error bars represent the standard error of the mean. Common
688 letters do not indicate differences by the Tukey's HSD test at $P \leqslant 0.05$.

689

690



691

692 **Fig. 2.** Chemical soil properties measured before the incubation assays. Inorganic N (IN),
693 dissolved organic (DOC), dissolved organic N (DON), and microbial biomass C (MBC)
694 affected by a corrective × fertilizer × layer interaction (A, B, C, and D, respectively); and
695 microbial biomass N (MBN) affected by the main effect of corrective and by a fertilizer ×
696 layer interaction (E and F, respectively). Only significant effects (Table 1) were shown. The
697 error bars represent the standard error of the mean. Common letters do not indicate
698 differences by the Tukey's HSD test at $P \leqslant 0.05$.

699

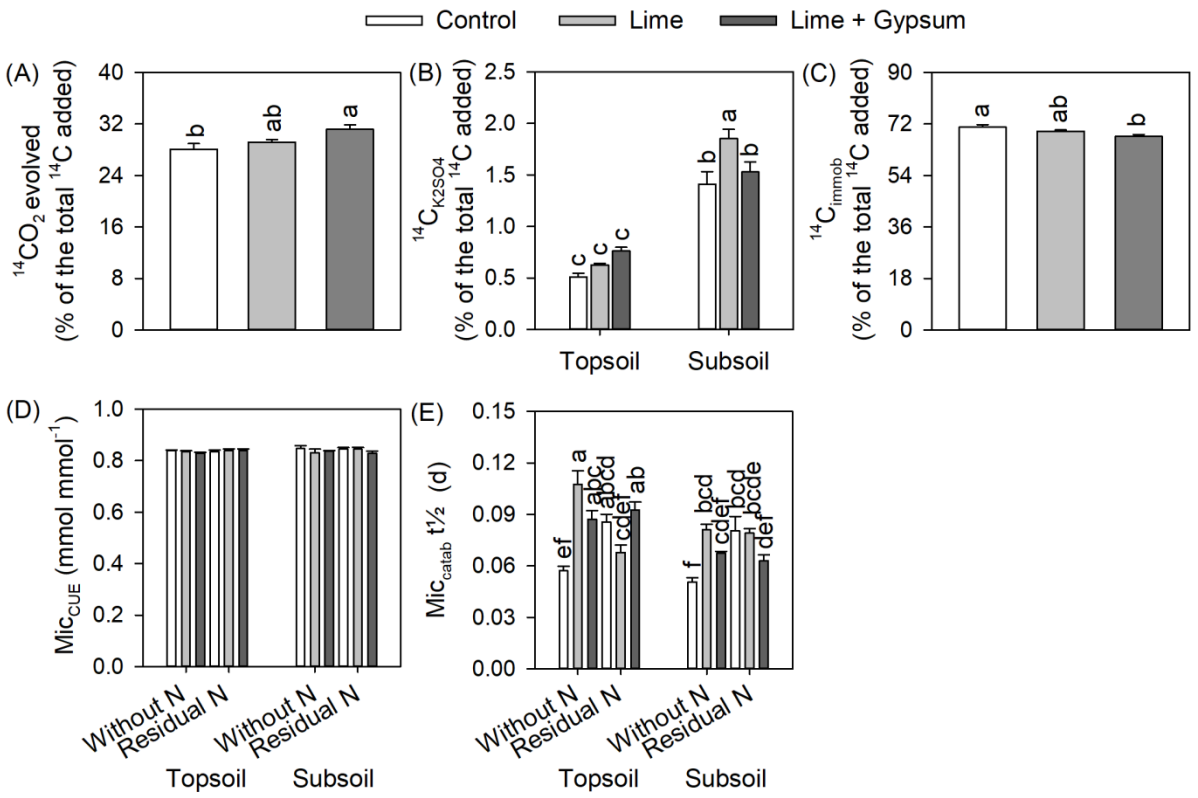


Fig. 3. Microbial and chemical parameters obtained at the end (30 d) of the ^{14}C–labeled glucose assay. $^{14}CO_2$ evolved from the soil affected by the main effect of corrective (A); ^{14}C recovered in the soil solution ($^{14}C_{K2SO4}$) affected by a corrective × layer interaction (B); ^{14}C immobilized into the microbial biomass ($^{14}C_{immob}$) affected by the main effect of corrective (C); and half–life of the first mineralizable pool (Mic_{catab} t½) affected by a corrective × fertilizer × 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 $P \leqslant 0.05$.

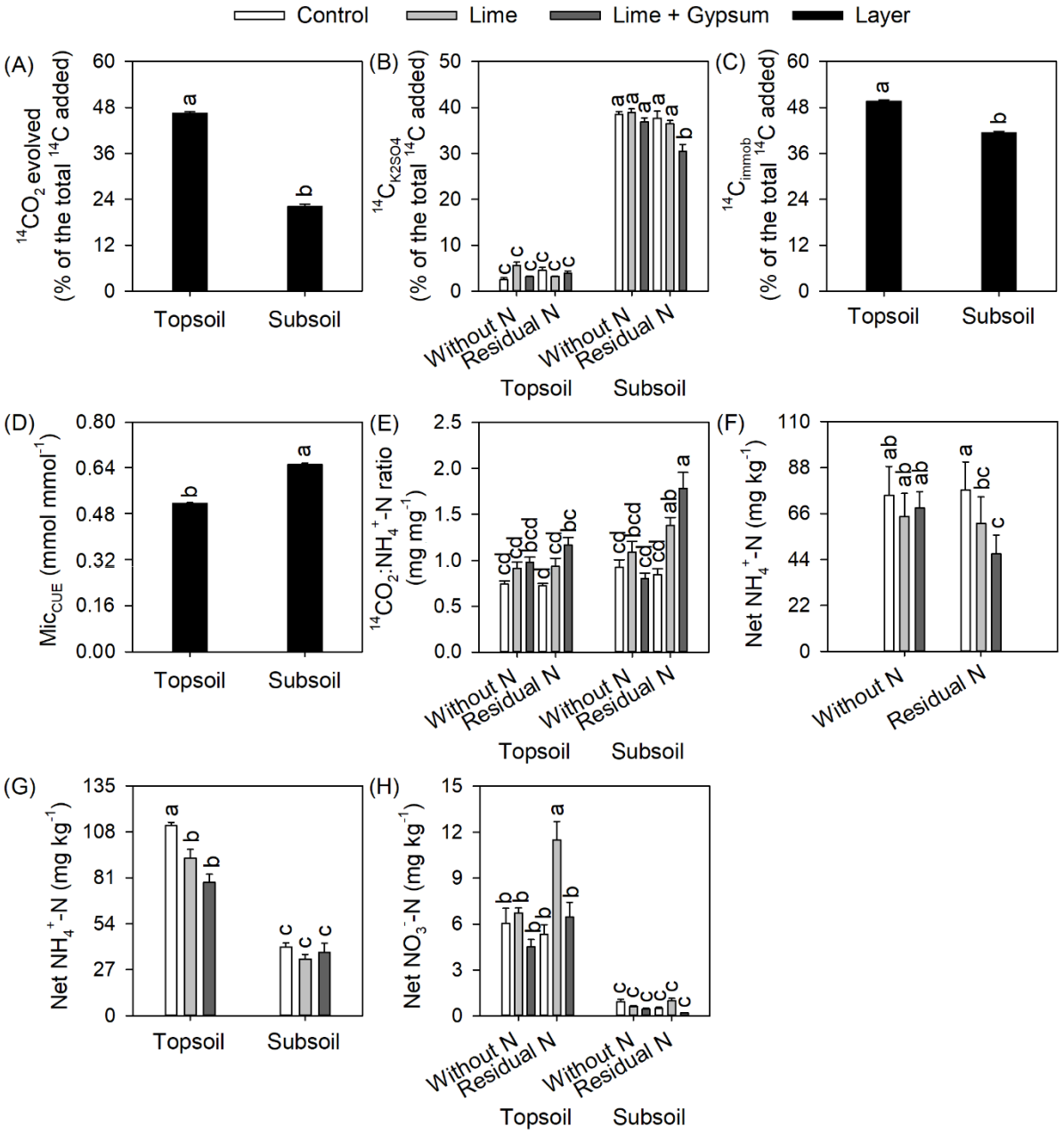


Fig. 4. Microbial and chemical parameters obtained at the end (48 h) of the ^{14}C–labeled arginine assay. $^{14}CO_2$ evolved from the soil affected by the main effect of layer (A); ^{14}C recovered in the soil solution ($^{14}C_{K2SO4}$) affected by a corrective × fertilizer × layer interaction (B); ^{14}C immobilized into the microbial biomass ($^{14}C_{immob}$) and microbial C use efficiency of the substrate (Mic_{CUE}) affected by the main effect of layer (C and D, respectively); the ratio between $^{14}CO_2$ evolved and NH_4^+–N in the soil ($^{14}CO_2$:NH_4^+–N) affected by a corrective × fertilizer × layer interaction (E); net NH_4^+–N production affected by corrective × fertilizer and corrective × layer interactions (F and G, respectively); and net

720 NO_3^-–N production affected by a corrective × fertilizer × layer interaction (H). Only
721 significant effects (Table 1) were shown. The error bars represent the standard error of the
722 mean. Common letters do not indicate differences by the Tukey's HSD test at $P \leqslant 0.05$.
723