

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

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1 Topsoil and subsoil C and N turnover are affected by superficial lime and

2 gypsum application in the short-term

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17 Abstract

18 Alleviation of subsoil acidity with lime or gypsum increases carbon (C) accumulation in deep 19 layers by stimulating root growth and C and nitrogen (N) inputs at depth. However, the 20 effects of these amendments combined with N fertilization on soil CO₂ emissions remain 21 controversial. We evaluated the effects of superficial lime and gypsum application and N-22 fertilizer on C and N dynamics and microbial C use efficiency (Mic_{CUE}) in samples taken 23 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 ¹⁴C-glucose and ¹⁴C-arginine to 24 25 assess C and N mineralization dynamics. Liming increased topsoil pH but had no effect on 26 subsoil acidity. Higher content of organic C, total N, and microbial biomass C and N were 27 found in the topsoil. The addition of soil corrective and N fertilizer had no effect on Miccue of added ¹⁴C–glucose. However, the Mic_{CUE} of ¹⁴C–arginine was <u>affected</u> only influenced by 28 29 the soil layer, and which was higherst in the subsoil. After the addition of arginine, net NH_4^+ -30 N production was highest in the topsoil control, while net NO₃⁻-N content was highest within the treatment lime + gypsum pluswith residual N in the same layer. We can conclude that 31 32 while lime and gypsum may ameliorate soil acidity, they have minimal effect on the C 33 cycling through the microbial biomass, particularly in the subsoil. 34

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

39 1. Introduction

40 Soil is an important C sink, storing two thirds of the C in the terrestrial biosphere 41 (Lorenz and Lal, 2018);, however, it can also be responsible for emitting greenhouse gases, 42 such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), known to affect 43 global warming (Pachauri et al., 2014). Soil respiration is largely controlled by the activity of 44 microbial communities, which are typically limited by the availability of labile C and N 45 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 46 47 biomass in the subsoil, therefore suggesting a C-limited lifestyle (de Sosa et al., 2018; Jones 48 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 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 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

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 ₂ O) 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.

89 **2. Material and methods**

90 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.

98 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) 99 control (without application of correctives); (*ii*) 1.45 Mg ha⁻¹ yr⁻¹ of lime; and (*iii*) 1.45 Mg 100 $ha^{-1} yr^{-1}$ of lime + 1.05 Mg $ha^{-1} yr^{-1}$ of gypsum. The "fertilizer" factor was composed of (i) 0 101 kg ha⁻¹ yr⁻¹ of N (here defined as without N) and (*ii*) 160 kg ha⁻¹ yr⁻¹ of N (here considered 102 103 as residual N). The trial had been cropped with soybean [Glycine max (L.) Merrill] in the wet 104 summer (November-March), immediately followed by maize (Zea mays L.) intercropped 105 with Guinea grass (Megathyrsus maximus Jacq. cv Tanzânia) in the dry off-season. After the 106 maize harvest (August), Guinea grass remained in the field as a cover crop, with subsequent 107 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, 108 109 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 110 111 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 112

113 160 kg ha⁻¹ yr⁻¹ (30 kg ha⁻¹ at sowing and 130 kg ha⁻¹ top–dressed to the respective
114 treatments when maize plants were at the V4–V5 stage).

- 115
- 116 2.2. Soil sampling and sample preparation

In the second dry-season, with the presence of Guinea grass (August 2018), three 117 118 samples were randomly taken from each field plot from depths of 0-10 cm (topsoil) and 40-119 60 cm (subsoil) using a 5.5 cm i.d. stainless steel corer and combined into one composite 120 sample per soil layer per plot. The soil was passed through a 5 mm mesh to remove roots and 121 macrofauna. This mesh size was used to minimize disturbance to the microbial community 122 (Jones and Willet, 2006). Prior to experimentation, the soil water content was measured 123 gravimetrically (24 h at 105 °C), and the water-holding capacity (WHC) of each soil layer 124 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 125 126 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 (5 127 g) with 25 ml of 0.5 M K₂SO₄ (200 rev min⁻¹, 30 min), followed by storage of the extracts at 128 4 °C (Jones and Willet, 2006). 129

130

131 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 K₂SO₄ solution (1:5 soil-to-K₂SO₄ solution; w/v), and determined using a Multi N/C 3100[®] analyzer (Analytik 138 139 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 140 determined colorimetrically following the salicylic acid procedure of Mulvaney (2018) and 141 142 vanadate method of Miranda et al. (2001), respectively. Dissolved organic N (DON) was calculated as the difference between TDN and dissolved inorganic N ($NH_4^+-N + NO_3^--N$). 143 144 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

To determine the Mic_{CUE}, a trace amount of ¹⁴C–labeled glucose (10 nM) was added 150 151 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.

For each sample, 5 g of soil moist 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

161 into anabolic (*i.e.*, cell growth and maintenance) and catabolic processes (*i.e.*, respiration) 162 was investigated. Immediately after ¹⁴C–glucose addition, a polypropylene vial containing 1 163 ml of 1 M NaOH was placed above the soil to trap the evolved ¹⁴CO₂. The tubes were then 164 sealed and incubated at 20 °C in the dark to reflect field conditions. The alkaline traps were 165 changed after 1, 3, 6, 10, and 24 h, and then 2, 3, 5, 7, 9, 12, 15, 19, 24, and 30 d after ¹⁴C 166 addition. The amount of ¹⁴CO₂ in the NaOH traps was determined using Optiphase HiSafe 3 167 scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and a Wallac 1404 liquid

168 scintillation counter (Wallac EG&G, Milton Keynes, UK).

To determine how much ¹⁴C–glucose remained in the soil at the end of the incubation, the soils were extracted with 0.5 M K₂SO₄ 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 ¹⁴CO₂ determination. The remaining K₂SO₄ extract was used to determine the NH₄⁺–N and NO₃⁻–N content in the soil, as described earlier. Microbial immobilization of ¹⁴C–glucose (¹⁴C_{immob}) at the end of the

176
$${}^{14}C_{\text{immob}} = {}^{14}C_{\text{total}} - {}^{14}C_{\text{K}_2\text{SO}_4} - {}^{14}CO_2$$
 (Eqn. 1)

177 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 178 amount of ${}^{14}C$ recovered in the 0.5 M K₂SO₄ extract, and ${}^{14}CO_2$ is the total amount of ${}^{14}C$ 179 recovered as ${}^{14}CO_2$ evolved from the microbial biomass.

180 Microbial CUE for ¹⁴C–glucose was estimated through an exponential kinetic model,
181 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
- 183 Software Ltd., London, UK):

184
$${}^{l4}C_{\text{soil}} = (Mic_{\text{catab}} \times \exp^{-k_c \times t}) + (Mic_{\text{anab}} \times \exp^{-k_a \times t})$$
 (Eqn. 2)

185	where ${}^{14}C_{\text{soil}}$ is the amount of ${}^{14}C$ remaining in the soil over time (<i>t</i>), <i>Mic</i> _{catab} and <i>Mic</i> _a	ınab			
186	describe the amount of ¹⁴ C partitioned into the catabolic and anabolic C pools and k_c	and k_a			
187	correspond to the exponential decay constants describing the turnover of the Mic_{catab}	ind			
188	Mic_{anab} pools, respectively. The first pool (Mic_{catab}) corresponds to a C pool which is rapidly				
189	used for catabolic processes, while the second pool (Mic_{anab}) constitutes the remaining C				
190	immobilized within the microbial biomass (i.e., used for cell growth and maintenance	, and			
191	ultimately necromass turnover) (Glanville et al., 2016). From this, Mic _{CUE} can be cale	culated			
192	as follows (Jones et al., 2018c):				
193	$Mic_{CUE} = Mic_{anab} / (Mic_{catab} + Mic_{anab})$	(Eqn. 3)			
194	The half–life for the first mineralizable pool ($Mic_{catab} t_{\frac{1}{2}}$) can be calculated from:				

195
$$Mic_{catab} t_{2} = ln(2) / k_{c}$$
(Eqn. 4)

197 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 198 very low C:N ratio of arginine (1.5:1) is much lower than the microbial biomass (C:N 7:1) 199 200 which means that the use of arginine as a sole C source leads to the excretion of excess NH4⁺ 201 from the microbial biomass (Bonde et al., 2001; Fujii et al., 2018). Briefly, 5 g of moist soil was placed in 50 cm³ polypropylene tubes to which 0.25 ml of ¹⁴C-labeled arginine was 202 added to the soil surface (50 mM; 3.35 kBq ml⁻¹; 140 mg N kg⁻¹ soil, 180 mg C kg⁻¹; 203 204 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 ¹⁴CO₂, and the tubes were 205 206 sealed and incubated at 20 °C. The alkaline traps were changed after 1, 3, 6, 9, 24, and 48 h following ¹⁴C–substrate addition. The amount of ¹⁴CO₂ in the NaOH traps was determined as 207 208 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 ¹⁴C-209

arginine sorbed on the solid phase or in soil solution was extracted with 0.5 M K₂SO₄

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):

216
$$Mic_{CUE} = {}^{14}C_{immob} / ({}^{14}C_{immob} + {}^{14}CO_2)$$
 (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 K₂SO₄ 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.

224

225 2.6. Statistical analysis

226 All statistical procedures were performed in R (version 3.6.3, The R Foundation for 227 Statistical Computing, Boston, MA, USA) with RStudio (v1.2.5019, RStudio, Vienna, 228 Austria) as front-end. The normality and homoscedasticity of the data were analyzed by 229 Shapiro–Wilk and Bartllet tests, respectively. Non–normally distributed data were log₁₀– transformed prior to analysis (*i.e.*, EC, OC, IN, MBC, DON, and net NH₄⁺–N and NO₃⁻–N 230 231 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 imes232 233 fertilizer \times soil depth) using a generalized linear model was carried out to assess selected

soil properties and parameters derived from soil incubation with ¹⁴C–glucose and ¹⁴C–

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 < 0.050unless otherwise stated.

238

3. Results

240 *3.1. Soil characteristics*

241 The pH results showed had an interaction of between the corrective with the and soil 242 layer, as well as ofbetween N fertilizer with theand soil layer (Table 1). In particular, pH in 243 the amended treatments was ~1.2 unit greater than the control infor the topsoil only (Fig 1A). 244 Further, soil pH control treatment was similar in both the topsoil and subsoil in the control 245 treatment, and was also similar in the subsoil ofto those treatments that receivinged lime and lime + gypsum in the subsoil (Fig 1A). In addition, residual N decreased 0.7 unit of the pH in 246 247 the topsoil, while in the subsoil showed, with the lowest values, with there was no effect of N 248 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 249 150 μ S cm⁻¹, respectively; Fig 1C). The application of lime + gypsum with N addition 250 251 increased the EC in the subsoil relative to the unamended control (regardless of the N 252 management) and lime without N (Fig 1C).

Organic C <u>was affected interactively byhad an interaction between</u> 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).

263 A three-way interaction was observed for iInorganic N, DOC, DON, and MBC had a 264 three way interaction (Table 1). Inorganic N was higher in the control with residual N 265 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 266 lower content in this layer relative to the topsoil (Fig. 2A). For DOC, the treatments lime + 267 268 gypsum without N in the topsoil and control without N in the subsoil were higher than lime + 269 gypsum with residual N in the topsoil (Fig. 2B). Overall, the DOC content in the topsoil and 270 subsoil were similar (Fig. 2B). The control (regardless of the N management) in the subsoil 271 had higher DON in the subsoil content compared with all topsoil treatments, regardless of the 272 N management, (which had overall lowest values) and lime + gypsum with residual N -in the 273 subsoil (Fig. 2C). The MBC content was higher in the control and lime + gypsum with 274 residual N in the topsoil compared in comparison with lime + gypsum without N in the same 275 layer and all treatments in the subsoil, with exception of theto control with N (Fig. 2D). 276 Microbial biomass-N was affected by presented the main effect of the correctives (Table 1), 277 where lime + gypsum was 15% higher, on average, compared with lime alone and the 278 unamended control (Fig. 2E). In addition, a fertilizer × layer interaction was also found 279 (Table 1), where MBN content was higher without N compared withrelative to residual N 280 addition in the topsoil, while the subsoil showedhad the lowest values and was not affected by N management (Fig. 2F). 281

The main effect of corrective was observed for ¹⁴CO₂ evolved from the soil (Table 1), 284 which had the following trend: lime + gypsum > lime > control, where 28-31% of the total 285 ¹⁴C-labeled glucose added to the soil was respired by microbes (Fig. 3A). In addition, less 286 than 2% of the glucose-derived ¹⁴C was recovered in the K₂SO₄ solution (*i.e.*, the proportion 287 of ¹⁴C sorbed to the solid phase or available in the soil solution) at the end of the incubation 288 289 period, with a higher amount observed in the subsoil than the topsoil for the corrective \times 290 layer interaction (Table 1; Fig. 3B). While no differences were observed in the topsoil, the 291 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 ¹⁴CO₂ evolved, but 292 with an opposite trend, where a higher amount of ¹⁴C was immobilized by microbes in the 293 294 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 295 296 mmol^{-1} (Fig. 3D). Moreover, a low amount (16%, representing the mean value across all treatments) of added ¹⁴C–glucose was rapidly respired by soil microbes (*i.e.*, Mic_{catab} pool), 297 while the majority of the ¹⁴C (82%, mean value across all treatments) was incorporated into 298 299 the microbial biomass (*i.e.*, Micanab pool) before its partial mineralization as CO₂ (data not 300 shown). However, Mic_{catab} t_{1/2} was influenced by a three-way interaction (Table 1). In this 301 regard, the unamended control without N addition in the subsoil had a lower Miccatab ty/2 302 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). 303

304

305 *3.3. Microbial arginine mineralization and carbon use efficiency*

The main effect of soil layer influenced the ¹⁴C–arginine mineralization (Table 1), which was much higher in the topsoil than in the subsoil, where 47% and 22% of the total ¹⁴C 308 added was evolved as CO2 after 48 h, respectively (Fig. 4A). The amount of arginine-derived 309 ¹⁴C recovered in the K₂SO₄ solution had a corrective \times fertilizer \times layer interaction (Table 1). 310 Overall, 32% more ${}^{14}C_{K2SO4}$ was recovered from the subsoil in comparison to the topsoil 311 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 312 addition, both ${}^{14}C_{immob}$ and Mic_{CUE} were influenced by the main effect of layer, similar to 313 ¹⁴CO₂ (Table 1). Thus, a higher amount (22%) of ¹⁴C–arginine was immobilized by microbes 314 315 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 ¹⁴CO₂:NH₄⁺–N ratio, which could be used to decouple C and N 316 317 utilization and mineralization of N compounds (arginine, in this case), was affected by a 318 three-way interaction (Table1), where lime + gypsum with N in the subsoil had a higher ratio 319 comparedin comparison with the otherremaining treatments, except lime alone with residual N in the same layer (Fig. 4E). With regard to the net NH₄⁺–N in the soil following arginine 320 321 addition, two interactions were observed, as follows: corrective \times fertilizer and corrective \times layer (Table 1). Under residual N addition, NH4⁺-N content in the control was higher 322 323 compared with lime and lime + gypsum, while treatments without N were similar between 324 them (Fig. 4F). Without any difference among treatments, the subsoil had lower net NH₄⁺–N content relative to the topsoil, which presented the following trend: control > lime \approx lime + 325 gypsum, with values in the range of 78–111 mg kg⁻¹ (Fig. 4G). Lastly, the net NO₃⁻–N was 326 327 affected by a three–way interaction (Table 1). With a pattern comparable to the NH₄⁺–N 328 dynamics, the subsoil had much lower NO₃⁻–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 329 330 treatments (Fig. 4H). In the topsoil, however, the lime-treated soil with residual N presented 331 the highest NO₃⁻–N content, while the remaining treatments did not differ from each other (Fig. 4H). 332

334 4. Discussion

335 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 336 337 similar to the response reported by Inagaki et al. (2017), who found a decrease in the soil 338 acidity in the topsoil but not in the subsoil, with no effect of gypsum. Garbuio et al. (2011) 339 also observed a relative increase in soil pH down to 20 cm in a no-till system, 30 mo after 340 lime addition. The addition of N fertilizer also lowered topsoil pH, which we attribute to the 341 application of NH₄⁺-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 342 343 observe an increase in EC for the treatment with lime + gypsum coupled to residual N 344 compared with control withtout N and lime, irrespective of the N management. We attribute 345 this to the addition of gypsum, which promotes the vertical displacement of ions to deep 346 horizons (Ritchey et al., 1980), including those derived from the N fertilizer.

347 Organic C and TN concentrations were higher in the topsoil relative to the subsoil. 348 We ascribe this to the higher root mass in this layer and the deposition of plant residues on 349 the soil surface. While C and N inputs on topsoil are derived from shoot residues and 350 fertilizer addition, subsoil inputs are largely root-derived and tend to be less labile, and 351 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 352 353 al., 2014), stimulation of soil microbial activity, and solubilization of organic matter (Carmeis 354 Filho et al., 2017; Inagaki et al., 2017; Vieira Fontoura et al., 2019). However, adequate 355 management of pH and base cation correctives have the potential to enhance C storage in 356 soils, directly through enhanced plant growth or indirectly by reducing soil erosion (Carmeis 357 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 \pm 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 \times 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 NH₄⁺-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 CO₂ 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 ¹⁴C–

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

385 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 386 387 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

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 ${}^{14}CO_2$ 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.*, ¹⁴CO₂ evolved and ¹⁴C_{immob}) was observed in response to lime and gypsum application. 408 This suggests that the microbial community structure has not changed greatly in response to 409 410 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 411 substrate-derived CO₂ from a strongly acid (pH of 4.1) in comparison with a slightly acid soil 412 413 (pH of 6.6). Moreover, Kemmitt et al. (2006) reported no difference in the mineralization as of ¹⁴C–glucose for soil with different pH values (3.5–6.8). Our findings support the above are 414 congruent with the above studies. In addition, the overall higher ${}^{14}C_{K2SO4}$ in the subsoil is 415 416 likely explained by the lower microbial activity in this soil layer relative to the topsoil, where 417 MBC and MBN are higher.

418 The Mic_{CUE} for ¹⁴C–glucose averaged 0.84, which is considered normal to high since 419 the average value for sugars in the soil is 0.68, but ranging from 0.25 to 0.95 (Jones et al., 420 2018a; Jones et al., 2019). Comparing 970 agricultural soils from Australia, Jones et al. 421 (2019) observed a progressive decline in Mic_{CUE} with increasing soil acidity at pH values 422 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 Miccue declined [i.e., pH of 5.5, as reported by Jones et al. 423 (2019)], we found no difference among treatments. In general, the ¹⁴C–glucose presented a 424 425 rapid turnover, with Mic_{catab} $t_{\frac{1}{2}} < 0.5$ d, suggesting a slightly high C flux through the soil 426 solution, in comparison to the subsequent flux of substrate-derived C through the biomass 427 (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 428 429 the subsoil, suggesting that catabolic processes were slower in the absence of soil correctives. 430 This can be a result of indirect acidity stress such as Al toxicity, which is known to interfere 431 with some respiratory pathways, requiring a re-routing of the metabolism and leading into 432 more inefficient C pathways (Jones et al., 2019).

434	4.3. Carbon partitioning and microbial carbon use efficiency in response to arginine
435	Unlike ¹⁴ C–glucose, the turnover of ¹⁴ C–arginine was different between the two
436	layers, with higher ${}^{14}CO_2$ respired and overall much lower amounts of ${}^{14}C-K_2SO_4$ in the
437	topsoil. In general, different substrates lead to different Miccue values (Jones et al., 2018a),
438	with a higher Mic_{CUE} for sugars relative to amino acids (~0.68 and ~0.55, respectively). Jones
439	et al. (2018b) reported a decrease in 14 CO ₂ evolution in the first 6 h across soil depths, but
440	mineralization rates were similar after 48 h. Garbuio et al. (2011) observed higher ${}^{14}CO_2$
441	evolved in surface soil up to 24 h following ¹⁴ C-amino acid mix (containing equimolar
442	proportions to give an individual amino acid concentration of 666 μ mol L ⁻¹ and specific
443	activity of 1.0 kBq mL^{-1} : alanine, arginine, aspartic acid, glutamic acid, glycine, histidine,
444	isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine)
445	addition in lime-treated soils. However, after 48 h, there was no difference for arginine-
446	derived ¹⁴ CO ₂ between soil correctives nor fertilizer management, regardless of the soil layer.
447	Short-term incubation assay of arginine (from 24 h to 96 h) is generally used to provide
448	information on enzyme (e.g., arginase) activity in microbial communities and is strongly
449	influenced by soil C:N ratios (Fujii et al., 2019). On temperate acid-forests, Fujii et al. (2020)
450	observed a decrease in arginine mineralization (as ¹⁴ CO ₂ evolution) with increasing soil C:N
451	ratio (C:N > 20), and a greater flux with increasing soil pH. However, C:N ratio was beyond
452	values pointed by Fujii et al. (2020) for both soil layers (9.4 in the topsoil and 8.3 in the
453	subsoil), and the ¹⁴ CO ₂ evolution from arginine was higher in the topsoil, where the C:N ratio
454	was higher. In contrast to Fujii et al. (2020) and Jones et al. (2018b), Bonde et al. (2001)
455	reported a positive correlation of arginine ammonification with microbial biomass and
456	microbial activity. Evaluating the long-term effect of lime and N fertilizer application on
457	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, phenylalanine, proline, serine, threonine, tyrosine, and valine; final concentration 5 mM) 459 turnover in agricultural soils. Jones et al. (2005) observed no effect on ¹⁴CO₂ evolved 460 following N fertilizer application, while soil pH slightly decreased. The authors concluded 461 that total microbial activity is likely a key determinant of amino acid turnover since 462 463 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 464 465 arable; and drainage) have shown little effect on topsoil. Therefore, the higher MBC and MBN in the topsoil could be related to ¹⁴C–arginine turnover response. 466

The Mic_{CUE} for ¹⁴C–arginine had an opposite trend from its turnover, with the highest 467 468 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, the higher Mic_{CUE} in the subsoil is likely explained by the lower microbial biomass turnover 470 471 in this layer, which is supported by the higher amount of ${}^{14}C$ -arginine immobilized and much lower ${}^{14}C_{K2SO4}$ recovered in the topsoil than in the subsoil. However, the ${}^{14}CO_2$:NH₄⁺–N ratio 472 was similar between the topsoil and subsoil. The treatment lime + gypsum with residual N 473 had the lowest efficiency regarding ¹⁴C-arginine mineralization, with a higher proportion of 474 ¹⁴CO₂ evolved per molecule of NH₄⁺–N mineralized. Evaluating the selective N 475 476 mineralization relative to C from arginine, Fujii et al. (2020) reported ¹⁴CO₂:NH₄⁺–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 477 48-h incubation period. Moreover, Fujii et al., 2020 suggest that C and N mineralization was 478 decoupled for treatments with the highest ¹⁴CO₂:NH₄⁺–N ratio (1.5), indicating differences in 479 480 intercellular activities in the arginine degradation into urea (CH₄N₂O) and ornithine $(C_5H_{12}N_2O_2)$. Since there was no difference for Mic_{CUE} of ¹⁴C-arginine related to corrective 481 482 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).

485 Despite the lower net NH_4^+ -N production with lime and lime + gypsum compared with the control in the topsoil, the highest net NO₃⁻–N content occurred for lime with residual 486 N fertilizer in the same layer. Our results partly agree with Garbuio et al. (2011), who 487 488 observed higher N mineralization and nitrification under liming. A positive correlation 489 between soil pH and arginine ammonification was also obtained by Fujii et al. (2020). In 490 addition, Garbuio et al. (2011) observed that N fertilizer application did not affect the ¹⁴C 491 mineralization of the amino acid mix, but enhanced nitrification rates with increasing soil 492 depth. In general, increased availability of inorganic N does not repress microbial amino acid 493 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 494 495 production of mineral N in the subsoil compared with the topsoil suggests a slower arginine 496 degradation in the deep layer. In addition, Jones et al. (2018b) reported higher net NH_4^+ –N 497 production following arginine addition, suggesting that the subsoil microbial activity is more 498 C-limited rather than by N. Although the lower arginine mineralization in the subsoil, DON 499 concentration was high in this layer, suggesting an effect of repressed enzyme activity (Fujii 500 et al., 2020) since DON compounds are preferred N sources by microorganisms in 501 comparison to NO_3^- (Abaas et al., 2012; Jones et al., 2018b)

502

503 **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 active. On the other hand, the Mic_{CUE} of ¹⁴C-arginine is only affected by the soil layer, where 509 510 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 soil pH, the main response to lime and/or gypsum, or with the C:N ratio, as previously 512 513 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 CO₂ to the 519 atmosphere.

520

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665 **Table**

666

667 **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 ${}^{14}C-$

670	labeled tracers.	<i>P</i> -values	<0.050 a	re highlighte	d in	bold.
				0 0		

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 _{1/2}	<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; ¹⁴CO₂: ¹⁴CO₂ evolved from the soil;

673 ¹⁴C_{K2SO4}: ¹⁴C recovered in the soil solution; ¹⁴C_{immob}: ¹⁴C immobilized into the microbial biomass; Miccue: microbial C use

 $674 \qquad \text{efficiency of the substrate; Mic_{catab} t_{2}: half-life of the first mineralizable pool; {}^{14}\text{CO}_2: NH_4^+ - N: \text{ the ratio between } {}^{14}$

- evolved and NH₄⁺-N in the soil at the end of the incubation; Net NH₄⁺-N: net NH₄⁺-N production at the end of the
- incubation; Net NO₃⁻-N: net NO₃⁻-N production at the end of the incubation.

679 Figure captions







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Fig. 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 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 $P \le 0.05$.



Fig. 3. Microbial and chemical parameters obtained at the end (30 d) of the 14 C–labeled 701 702 glucose assay. ¹⁴CO₂ evolved from the soil affected by the main effect of corrective (A); ¹⁴C recovered in the soil solution ($^{14}C_{K2SO4}$) affected by a corrective × layer interaction (B); ^{14}C 703 immobilized into the microbial biomass (¹⁴C_{immob}) affected by the main effect of corrective 704 705 (C); and half–life of the first mineralizable pool (Mic_{catab} $t^{1/2}$) affected by a corrective × 706 fertilizer \times layer interaction (E). Only significant effects (Table 1) were shown, except for the 707 microbial C use efficiency of the substrate (Mic_{CUE}), where no effect was observed but all 708 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 $P \le 0.05$. 709



Fig. 4. Microbial and chemical parameters obtained at the end (48 h) of the ¹⁴C–labeled arginine assay. ¹⁴CO₂ evolved from the soil affected by the main effect of layer (A); ¹⁴C recovered in the soil solution (¹⁴C_{K2SO4}) affected by a corrective × fertilizer × layer interaction (B); ¹⁴C immobilized into the microbial biomass (¹⁴C_{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 NH₄⁺–N in the soil (${}^{14}CO_2$:NH₄⁺–N)

affected by a corrective \times fertilizer \times layer interaction (E); net NH₄⁺–N production affected

719 by corrective \times fertilizer and corrective \times layer interactions (F and G, respectively); and net

- 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 $P \le 0.05$.