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1 **Grazing, liming, and fertilization: shifts on soil fertility and microbial**
2 **community in a no-till sheep-soybean integrated system**

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18 **Grazing, liming, and fertilization: shifts on soil fertility and microbial**
19 **community in a no-till sheep-soybean integrated system**

20 **Abstract**

21 Integrated crop–livestock systems are recognized for intensifying food production as long
22 as adequate management of grazing, fertilization, and liming is practiced. There is still a
23 lack of information about the effect of these factors on the soil microbiological
24 community. This study evaluated the effects of grazing, liming, and different P and K
25 fertilization strategies on the soil microbial community and its relationship with soil
26 chemical properties. For this purpose, a field experiment was established in 2017 on a
27 subtropical Acrisol. Treatments consisted of two production systems: (i) integrated crop-
28 livestock system (with winter sheep grazing) and (ii) specialized system (ungrazed),
29 combined with two periods of P and K fertilization: (i) conventional fertilization (at
30 soybean planting) and (ii) system fertilization (in the Italian ryegrass pasture). The effect
31 of liming (with and without) was also evaluated. Eighteen months after lime application,
32 the soil was sampled in the 0–5 and 10–15 cm soil layers to evaluate soil chemical
33 properties (pH, exchangeable Al, Ca, and Mg, available P and K, cation saturation, and
34 Al saturation) and soil microbial community composition. In the surface soil layer, a
35 negative impact of lime application was observed for Gram+ bacteria (-29%) and
36 actinomycetes (-21%), consequently decreasing the total bacterial community (-26%) and
37 total microbial biomass (-27%). This negative impact of liming on Gram+ bacteria and
38 actinomycetes was negatively related to the increase in soil pH, exchangeable Ca and Mg
39 concentrations, and positively related to Al saturation and available K. The combination
40 of an integrated system or system fertilization with lime increased arbuscular mycorrhizal
41 fungi community 3.0-fold compared to a specialized system or conventional fertilization
42 in the subsurface soil layer. In addition, sheep grazing increased saprophytic fungi
43 biomass by 50% in the subsurface soil layer compared to the specialized system. These
44 positive impacts on the soil fungal community were probably associated with increased
45 plant residue input. Sheep grazing and the fertilization strategy did not affect soil
46 chemical properties or the microbial community at the soil surface. Liming, system
47 fertilization, and sheep grazing benefitted the soil fungal community, thus improving soil
48 health in highly weathered agricultural soils.

49 **Keywords:** soil microbial community, phosphorus, potassium, lime, crop-livestock
50 systems.

51 **1. Introduction**

52 Integrated crop-livestock systems (ICLS) are defined as systems where temporary
53 rotation or alternating cash crops with pastures over time (Moraes et al., 2014) and are
54 envisioned as routes for sustainable intensification with economic viability for food
55 production (Russelle et al., 2007; Allen et al., 2007; Lemaire et al., 2014). The ICLS has
56 several benefits as they ensure high crop yields, diversifying farmers' income while
57 preserving natural resources, and contributing to the agricultural sector's climate and
58 economic resilience (Peyraud et al., 2014; Peterson et al., 2020). In addition to sustainable
59 intensification of production systems, ICLS is recognized for improving soil health
60 (Sanderson et al., 2013; Garrett et al., 2017; Valani et al., 2020). The main reasons for the
61 improvements in soil quality are: i) increased carbon (C) inputs (Sá et al., 2017; Luz et
62 al., 2019; Brewer and Gaudin, 2020); ii) increased labile C by organic root exudates from
63 pasture and animal manure, favoring soil microbial diversity and activity (Bustamente et
64 al., 2011; Sandhu et al., 2019) and improved soil aggregation, nutrient cycling, and soil
65 health (Sekaran et al., 2021). The soil microbial community influences soil functionality
66 through its role in nutrient cycling, mineralization, and stabilization of soil organic carbon
67 (SOC) (Sekaran et al., 2019).

68 In ICLS, the animal grazing component induces greater root growth of pastures
69 and can positively affect the soil microbial community (López-Mársico et al., 2015).
70 Besides grazing, other practices, such as liming, can impact the soil microbial community.
71 Soil pH is critical for the soil microbial community (Wu et al., 2017; Wan et al., 2020).
72 Bacterial and fungal populations are affected by soil pH. Gram-positive (Gram+) bacteria
73 and fungi appear to be more tolerant to low soil pH, high aluminum (Al) concentrations,
74 and low C inputs than Gram-negative (Gram-) bacteria (Pietri & Brookes, 2009). In
75 addition to the direct effects of improving soil chemical properties in acid soils (Holland

76 et al., 2018; Hale et al., 2020), liming contributes to higher root and shoot biomass
77 production due to a better environment for plant growth (Alves et al., 2021), as well as
78 providing a better environment for the soil microbial community (Treonis et al., 2004;
79 Sen et al., 2022). However, the impact of liming on the soil microbial community in
80 acidic, highly weathered agricultural soils is limited.

81 Recently, alternative fertilization strategies with phosphorus (P) and potassium
82 (K) in ICLS in tropical and subtropical regions have addressed productive concerns, both
83 of cash crops and pastures for animal feed, and the impacts on soil fertility and health
84 (Farias et al., 2020; Alves et al., 2022, Pires et al., 2023). The advances are related to the
85 system fertilization strategy. This new fertilization strategy takes advantage of nutrient
86 cycling within an agroecosystem for maximum yield of each nutrient (Farias et al., 2023).
87 For soil with available P and K above the critical level (CQFS-RS/SC 2016), P and K
88 fertilization occurs on the pasture phase in ICLS (system fertilization), instead at spring
89 crop planting ("conventional fertilization") (Alves et al., 2021). The P and K fertilization
90 for the pasture phase promotes greater pasture and animal production without diminishing
91 the cash crop (soybean) yield (Farias et al., 2020; Alves et al., 2022). The higher pasture
92 production and animal manure waste increases C input into the soil, promoting a better
93 environment for the soil microbial community. However, because this is a new strategy,
94 its impacts on microbial community structure are poorly known.

95 To understand how the microbial community and soil chemical properties are
96 altered by fertilization and soil acidity correction in ICLS, we evaluated the effects of
97 sheep grazing, liming, and P and K fertilization strategies in a field experiment under no-
98 till ICLS in a subtropical Brazilian region. We hypothesize that no-till ICLS conducted
99 under system fertilization provided better soil conditions for the soil microbial
100 community and liming increased the bacteria and fungi biomass. Our findings will

101 provide an important foundation to guide fertilization and soil acidity correction strategies
102 in ICLS.

103 **2. Material and methods**

104 *2.1. Site description and experimental design*

105 The study site was located at the Experimental Agronomic Station of the Federal
106 University of Rio Grande do Sul, in Eldorado do Sul, Rio Grande do Sul State, Brazil
107 (30°05' S, 51°39' W, 46 m above sea level). The regional climate is humid subtropical
108 (Cfa), according to the Köppen classification (Alvares et al., 2013), the regional climate
109 is humid subtropical (Cfa), with mean annual air temperature and precipitation of 19.4 °C
110 and 1440 mm, respectively. The soil was classified as Acrisol (FAO, 2015). Soil chemical
111 and physical properties of the study site are described in **Table 1**.

112 The experiment was established in an area of 4.8 ha split into 16 paddocks of 0.23
113 to 0.41 ha. The experimental design was a randomized complete block (RCBD) with 4
114 replications in a 2 × 2 factorial system and split-plots. The first factor was animal grazing,
115 that is, whether the cover crop was grazed (integrated system) or ungrazed (specialized
116 system). The second factor was the P and K fertilization strategy, which involved fertilizer
117 in the soybean cropping phase (conventional fertilization) or winter pasture (Italian
118 ryegrass - *Lolium multiflorum*) phase (system fertilization).

119 The split-plot was the effect of lime application (i.e., surface liming and a control
120 with no lime). The effect of soil acidity neutralization (i.e., with or without lime) was
121 examined in all plots by excluding an area of 32 m² (4 m × 8 m) from lime application to
122 maintain the original acidity conditions. Soil acidity was amended by applying lime in
123 the amount needed to raise pH to 6.0 according to regional recommendations (CQFS-
124 RS/SC, 2016). The soil was limed with 7.5 Mg ha⁻¹ of dolomitic lime [CaMg(CO₃)₂] with

125 an effective neutralizing power of 72%. Liming was applied in July 2017 without
126 incorporation.

127 The sheep grazing started in June and finished in October in 2017 and 2018 (**Table**
128 **2**). Italian ryegrass was established by broadcasting viable seeds at a rate of 25 kg ha⁻¹
129 with a centrifugal distributor in May of each year. Urea (45% N) was applied at 150 kg
130 N ha⁻¹ to all paddocks at the Italian ryegrass V3 stage (3 totally expanded leaves). **Table**
131 **2** contains detailed information about the animals. A variable number of put-and-take
132 animals (Mott and Lucas, 1952) was used to maintain the desired sward canopy height
133 (SCH). This method was used to adjust the stocking rate and maintain the average SCH
134 at 15 cm, which provides the optimum plant structure for maximizing animal production
135 (Carvalho, 2013). The SCH was monitored at 7-day intervals by using a sward stick
136 (Barthram, 1985) for the measurement of 150 randomly chosen points per experimental
137 unit monthly. At the end of the pasture phase, residual ryegrass was terminated with
138 glyphosate herbicide before soybean planting.

139 The P and K fertilizer rates were calculated according to CQFS-RS/SC (2016),
140 which considers that when soil P and K levels are above high, only replace nutrients
141 removed. The rates were calculated from the amounts of P and K removed by soybean
142 with an expected yield of 4.0 Mg ha⁻¹ (Table 2). Before soybean planting, seeds were
143 inoculated with *Bradyrhizobium* strains at a dose of 400 ml for every 100 kg of seed. The
144 commercial product was Gelfix 5® with a guarantee of 5 × 10⁹ colony-forming units of
145 *Bradyrhizobium japonicum* per mL. Properly inoculated soybean seeds do not require N
146 fertilization (CQFS-RS/SC, 2016). Soybean row spacing was 45 cm. Cultivars, planting
147 method, harvesting date, and planting density for the 2017/2018 and 2018/2019 seasons
148 are presented in **Table 2**.

149 2.2. *Soil chemical properties, total microbial biomass, and labile carbon analysis*

150 Soils were sampled in January 2019 in two layers (0–5 and 10–15 cm). Sub-samples were
151 packed in Styrofoam boxes with ice to preserve the samples. Without prior drying, the
152 soil samples were sent to the laboratory, freeze-dried, and analyzed for the soil microbial
153 community composition.

154 Another part of the soil samples was dried in a forced-air oven at 45 °C, the larger
155 lumps crumbled, ground, and sieved ($\text{\O} = 2.0 \text{ mm}$) for determination of soil chemical
156 properties. Soil pH was determined in water suspension at a 1:1 soil-water ratio in a
157 benchtop pH meter model Digimed DM-22®. Soil available P and K were extracted with
158 Mehlich-1 solution. Mehlich-P was analyzed colorimetry (Femto 600 Plus® colorimeter).
159 Mehlich-K was analyzed by emission (Digimed NK-2000® flame photometer).
160 Exchangeable calcium (Ca), magnesium (Mg), and Al were extracted with KCl 1.0 mol
161 L^{-1} , Ca and Mg analyzed AA spectrometry (PerkinElmer AAnalyst 200® atomic
162 absorption spectrometer). Extrable Al was analyzed by titration with NaOH 0.0125 mol
163 L^{-1} solution (Tedesco et al., 1995). The sum of exchangeable cations (SC) was
164 determined by summing Ca, Mg, and K. The cation exchange capacity at pH 7.0
165 ($\text{CEC}_{\text{pH}7.0}$) was calculated by $\text{SC} + (\text{H} + \text{Al})$. Cation saturation was calculated as $V (\%) =$
166 $(\text{SC}) / \text{CEC}_{\text{pH}7.0} \times 100$; and Al saturation as $m (\%) = [\text{Al} / (\text{Ca} + \text{Mg} + \text{K} + \text{Al})] \times 100$ (CQFS-
167 RS/SC, 2016).

168 The soil microbial community composition was assessed by analyzing the
169 phospholipid fatty acids (PLFA) composition, which was performed using freeze-dried
170 samples. Total lipids were extracted using a modification of the Bligh and Dyer (1959)
171 extraction (White and Rice, 2009). Total lipids were extracted by 10 mL of methanol
172 (25.0 mol L^{-1}), 5 mL of chloroform (12.0 mol L^{-1}), and 4 mL of phosphate buffer (0.5
173 mol L^{-1}) at pH 7.4 on 5 g freeze-dried soil. Nanopure water (5 mL) and chloroform (5
174 mL) were added after 3 hours to separate the mixture into polar and non-polar fractions,

175 whereas total lipids remained in the non-polar phase. Phospholipids were isolated from
176 neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable
177 BAKERBOND® SPE Columns, J.T. Baker®, Avantor, Radnor, Pennsylvania, USA) and
178 eluted with methanol. The phospholipids were then saponified by KOH, methylated to
179 fatty acid methyl esters (FAME), and analyzed by Thermo Scientific Trace GC-ISQ mass
180 spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a
181 DB5-MS column (30 m x 250 µm in diameter x 0.25 µm film thickness; Agilent
182 Technologies, Santa Clara, California, USA). Helium was used as carrier gas. FAME
183 peaks were recognized by different retention times in comparison with the bacterial acid
184 methyl esters mix (BAME; Matreya 1,114; Matreya LLC, Pleasant Gap, Pennsylvania,
185 USA).

186 Sample peaks were quantified based on a comparison of the abundance with an
187 internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g⁻¹ dry soil.
188 **Table 3** shows the groupings of fatty acids and their respective microbial groups. The
189 total microbial biomass estimate from PLFA was determined as the sum of Gram+,
190 Gram-, actinomycetes, saprophytic fungi, and arbuscular mycorrhizal fungi (AMF),
191 except nematodes and protozoa, which were not determined by the method.

192 Permanganate oxidible carbon (POxC), also known as active carbon, was
193 measured following the procedure of Weil et al. (2003). Briefly, 2.5 g soil was mixed
194 with 18 mL of deionized water and 2 mL of 0.2 M KMnO₄, then shaken for 2 min and
195 allowed to settle for 10 min. Following settling, 0.5 mL of the supernatant was transferred
196 into another tube and mixed with 49.5 mL of deionized water. Absorbance was measured
197 in a spectrophotometer at 555 nm. The change in KMnO₄ concentration was used to
198 estimate the amount of oxidized C, assuming that 1 mM KMnO₄ is consumed in the
199 oxidation of 0.75 mM or 9 mg of C.

200 2.3. *Statistical analysis*

201 For the obtained values of each chemical property and each microbial group, statistical
202 analyses were performed with the software SAS® 9.4 (SAS, 2015). The residuals were
203 tested for normality with the Shapiro–Wilk test and variance homoscedasticity with the
204 Levene test, both at a significance level of 5%, before analysis of variance (ANOVA, $p <$
205 0.05).

206 The sample size was 32, composed of with or without of liming (2), fertilization
207 strategies (2), presence and absence of grazing (2), and replicated in four blocks (4). The
208 effects included in the statistical model were fertilization strategy (conventional or system
209 fertilization), grazing (specialized or integrated system), and liming (with or without).
210 Fertilization strategy (F), grazing (G), liming (L), and the interactions F*G, F*L, G*L,
211 and F*G*L, were used as fixed effects, and block and its interactions as random effects.
212 We used the PROC MIXED procedure and RANDON effect in SAS® 9.4 (SAS, 2015).
213 When significant, differences between treatment means were evaluated with Tukey's test,
214 also at the 5% significance level.

215 Linear regression models were used to explore the relationships of soil pH, cation
216 saturation, Al saturation, exchangeable Ca and Mg, and available K with microbial
217 biomass, in addition to the relationships between pH, cation saturation, Al saturation,
218 exchangeable Ca and Mg with gram+ bacteria. Regression models were fitted using all
219 data, and the models were evaluated using statistical significance and the coefficient of
220 determination (R^2). Person's correlation coefficient was used to analyze the correlation
221 between soil chemical properties and microbial communities using the program R Core
222 Team (2013).

223 The soil chemical and microbial properties data were also submitted to
224 multivariate analysis by principal component analysis (PCA) using the R software, with
225 the Vegan statistical package (R Core Team, 2013).

226 3. Results

227 3.1. Soil chemical properties

228 Soil chemical properties changed as a function of soil liming (**Table 4**). Soil liming
229 increased soil pH from 4.5 to 5.5, exchangeable Ca content from 2.2 to 3.9 $\text{cmol}_c \text{ dm}^{-3}$,
230 exchangeable Mg content from 1.4 to 2.6 $\text{cmol}_c \text{ dm}^{-3}$, and cation saturation from 33
231 53% at 0-5 cm soil layer, compared to no lime application (**Table 5**). At the same layer,
232 lime application decreased the exchangeable Al from 0.8 to 0.2 $\text{cmol}_c \text{ dm}^{-3}$ and Al
233 saturation from 17 to 4%, compared to no lime (**Table 5**). On the other hand, at 10–15
234 only exchangeable Ca content was changed by liming. The limed treatment had an
235 exchangeable Ca content of 1.5 $\text{cmol}_c \text{ dm}^{-3}$ compared to 1.0 $\text{cmol}_c \text{ dm}^{-3}$ in the unlimed
236 treatment (**Table 5**). Fertilization strategy and sheep grazing did not change the evaluated
237 soil chemical properties.

238 3.2. Soil microbial community and labile carbon

239 The soil microbial community changed as a function of soil liming, sheep grazing, and
240 the interaction among lime, fertilization strategy, and sheep grazing. However, the liming
241 effect was restricted only to the 0–5 cm soil layer and did not influence the subsurface
242 10–15 cm soil layer (**Table 6**). Soil liming decreased total microbial biomass (–27%, from
243 45.5 to 33.2 nmol PLFA g^{-1} soil, **Fig. 1A**), the total bacterial community (–29%, from
244 20.7 to 14.6 nmol PLFA g^{-1} soil, **Fig. 1B**), Gram+ bacteria (–29%, from 14.7 to 10.4
245 nmol PLFA g^{-1} soil, **Fig. 1C**), and actinomycetes (–21%, from 2.4 to 1.9 nmol PLFA g^{-1}

246 soil, **Fig. 1D**), but increased the fungi:bacteria ratio (+17%, 0.15 to 0.18, **Fig. 1E**) at 0–5
247 cm soil layer.

248 Sheep grazing increased the saprophytic fungi community by 50% in the 10–15
249 cm soil layer (**Fig. 1F**). The interaction among sheep grazing, fertilization strategy, and
250 liming changed the AMF community at the 10–15 cm soil layer (**Table 7**). In the
251 integrated system with system fertilization, liming increased AMF 2.4 times compared to
252 the treatment without liming (1.2 and 0.5 nmol PLFA g⁻¹ soil, respectively). Furthermore,
253 at the 10-15 cm soil layer, the integrated system with liming and system fertilization
254 increased AMF by 3.0-fold compared to conventional fertilization (1.2 and 0.4 nmol
255 PLFA g⁻¹ soil, respectively). The combination of liming and system fertilization in the
256 integrated system increased the AMF community 3.0-fold compared to the specialized
257 system (1.2 and 0.4 nmol PLFA g⁻¹ soil, respectively). Other comparisons were not
258 significant (**Table 7**).

259 *3.3. Principal component analysis, Pearson's correlation, and the relationship* 260 *between soil chemical and biological properties*

261 The components PC1 and PC2 accounted for 72.7% of the total variation of soil microbial
262 community change at 0-5 cm soil layer; 48.3% of the variation was explained by PC1 and
263 another 24.4% was explained by PC2. The microbial groups were limited by soil pH, as
264 influenced by lime application or not. All microbial groups were positively correlated
265 with exchangeable Al content and Al saturation, and negatively correlated with pH, cation
266 saturation, and exchangeable Ca and Mg for the 0–5 cm layer (**Fig. 2**). The axes of the
267 components were not significant for the 10–15 cm layer, so we do not present PCA for
268 this layer.

269 These results were supported by the significant correlations between soil chemical
270 properties and the microbial community (**Fig. 3**). Pearson's correlation matrix highlighted

271 the positive and negative correlation between soil chemical properties and the soil
272 microbial community, which were distinct for the 0–5 and 10–15 cm soil layers. For the
273 surface (0–5 cm), soil microbial biomass decreased as a function of increasing pH ($r = -$
274 0.68), cation saturation ($r = -0.48$) and exchangeable Ca ($r = -0.44$) and Mg ($r = -0.59$)
275 concentrations and increased as a function of Al saturation ($r = 0.43$) and K availability
276 ($r = 0.68$) (**Fig. 3A**). In contrast, in the subsurface the microbial biomass increased as a
277 function of pH ($r = 0.41$), cation saturation ($r = 0.43$), and exchangeable Ca ($r = 0.41$) and
278 Mg ($r = 0.49$) concentration (**Fig. 3B**). Furthermore, at the 0–5 cm soil layer there was a
279 negative correlation between soil pH, cation saturation, and exchangeable Ca and Mg
280 concentration with Gram+ bacteria population (**Fig. 3A**). All these correlations were
281 significant ($p < 0.05$).

282 Similarly, at the 0–5 cm layer, we observed a negative relationship between soil
283 pH (**Fig. 4A**), cation saturation (**Fig. 4B**), exchangeable Ca (**Fig. 4D**) and Mg (**Fig. 4E**)
284 concentration, and positive for Al saturation (**Fig. 4C**) and available K contents (**Fig. 4F**)
285 with microbial biomass. There was a differentiation for the 10–15 cm layer, where soil
286 pH (**Fig. 4A**), cation saturation (**Fig. 4B**), exchangeable Ca (**Fig. 4D**) and Mg (**Fig. 4E**)
287 had a positive relationship with soil microbial biomass. Furthermore, a negative
288 relationship was observed between soil pH (**Fig. 5A**), cation saturation (**Fig. 5B**) and
289 exchangeable Ca (**Fig. 5C**) and Mg (**Fig. 5D**) concentration and Gram+ bacteria.

290 **4. Discussion**

291 Liming improved soil chemical properties by increasing pH, exchangeable Ca and Mg
292 content, and decreasing exchangeable Al, consequently affecting the soil microbial
293 community. Contrary to previous results studies (Kennedy et al., 2004; Xue et al., 2010;
294 Li et al., 2022), we found a negative effect of pH increase (**Fig. 4A**) on soil microbial
295 community at the surface soil layer, where lime application resulted in lower microbial

296 biomass (**Fig. 1A**), and total bacterial biomass (**Fig. 1B**). Although a modest stimulus in
297 the soil microbial community was noticed after lime application, the microbial biomass
298 did not change or even decreased due to the reduced of SOC after 15 days (Badalucco et
299 al, [1992](#); Wachendorf, [2015](#)). The decrease in total bacteria was mainly a result of the
300 decrease in Gram+ bacteria (**Fig. 1C**), which represents the largest portion of the total
301 bacteria and a large part of the total microbial biomass, along with actinomycetes (**Fig.**
302 **1D**). Our finding corroborates with previous studies, demonstrating a negative
303 relationship between soil pH and abundance of cy19:0, i16:0, i17:0, fatty acids respective
304 to Gram+ bacteria (Frostegård et al., [1993](#); Bååth and Anderson, [2003](#); Rousk et al.,
305 [2010a, b](#); Abalos et al., [2020](#)).

306 On the other hand, a positive relationship was observed between soil acidity-
307 related properties such as pH, cation saturation, Al saturation, and exchangeable Ca and
308 Mg with microbial biomass in the subsurface soil layer from 10–15 cm, differing from
309 the effect observed in the superficial 0–5 cm soil layer, which was decreased (**Fig. 4A, B,**
310 **C, D, and E**). These differing results observed between the surface (0–5 cm) and the
311 subsurface (10–15 cm) can be explained by the contribution of limestone to SOC
312 mineralization rate. Liming and subsequent elevation in soil pH, may indirectly increase
313 microbial activity initially by stimulating SOC decomposition (Andersson et al., [2000](#);
314 Pietri and Brookes, [2008](#); Garbuio et al., [2011](#)). A higher SOC mineralization rate was
315 observed in a soil incubation experiment (Xiao et al., [2018](#)), possibly linked to the
316 increased microbial activity due to CaCO₃ application, depleting the available substrate
317 and contributing to a gradual decrease in soil microbial biomass at the same level as the
318 control (Feng et al., [2016](#)). However, in our results, this effect was observed only at the
319 surface because the alkalizing effect of the lime was restricted to the first few
320 centimeters, not altering the subsurface (**Table 5**). Thus, we have a different behavior

321 between the surface and subsurface regarding soil microbial community and soil chemical
322 properties, as observed in **Fig. 3**.

323 Liming did not alter the two groups of fungi represented by the PLFA biomarkers
324 in the surface (0–5 cm of soil layer), suggesting that the fungal community was less
325 sensitive to soil pH changes. Our result were supported by Rousk et al. (2010a,b), who
326 observed a positive effect of increasing soil pH (from 4 to 8) in the soil microbial
327 community but no effect on the fungal community. A positive effect between lime
328 application, system fertilization, and grazing was observed on the AMF community in the
329 subsurface (10–15 cm of soil layer) (**Table 7**). This result can be likely due to AMF being
330 strongly influenced by plant-derived C (Nurlaeny et al. 1996; Tavi et al., 2013; Cui et al.,
331 2018), which was higher under lime versus without lime treatments (Nurlaeny et al. 1996;
332 Alves et al., 2021). In addition to liming, improved pasture nutrition with P and K
333 fertilizers to the pasture phase (system fertilization) promoted higher biomass production
334 of Italian ryegrass (Farias et al., 2020; Alves et al., 2022). Thus, the greater C inputs
335 through the contribution of pasture under system fertilization compared to conventional
336 fertilization likely favored the AMF community. In addition, in the integrated system, the
337 input of the animal manure and the induction of greater root growth by grazing (López-
338 Mársico et al., 2015) may provide a more suitable environment with higher organic C
339 source for fungal development for, both AMF and saprophytic fungi (**Fig. 1F**). This
340 increase in the fungal biomass in the subsurface play an important role in soil C
341 stabilization as they physically entangle micro-aggregates to form macro-aggregates, and
342 release cohesive substances such as glycoproteins (glomalin) that boosts aggregation
343 (Wilson et al., 2009; Veloso et al., 2020).

344 Fine roots and AMF often represent the largest C input into soils (Kramer et al.
345 2010; Clemmensen et al. 2013), and their fate is therefore of primary importance for SOC

346 balance, highlighting the importance of increasing the population of these organisms in
347 deeper soil layers for subsurface C increment. The AMFs are the dominant mycorrhizal
348 type in grasslands (Read and Perez-Moreno 2003), and their increase can be of interest,
349 especially in ICLS, since in these systems the soil was covered by grasses. Furthermore,
350 by moving nutrients from the soil to plant roots in exchange for photosynthate, AMF are
351 the agents directing C to the soil (Johnson et al. 2013). Besides better utilization of
352 mineral nutrients, the deposition of fungal material can increase soil aggregation that can
353 prevent the decomposition of soil organic matter (Wilson et al. 2009).

354 Song et al. (2018) demonstrated that microbial composition and diversity were
355 directly correlated with soil fertility, and evolving soil physical and chemical properties.
356 In particular, the diversity of the bacterial community is highly dependent on the nutrient
357 supply in soils. Soil pH, SOC, TN, available N, and available P contents are factors that
358 have a close relationship with the abundance of most bacterial groups, indicating that soil
359 nutrient availability and supply had great importance for bacterial community
360 maintenance (Li et al., 2014; Liu et al., 2014; Zhang et al., 2016). In our study, the main
361 changes in the microbial community resulted from the change in soil pH, as a result of
362 the lime application.

363 Although this study makes significant advances in understanding the effects of
364 liming and fertilization strategies in ICLS on microbial groups, more research should be
365 conducted to verify microbial groups at the species level, aiming at identifying the effects
366 of each factor on the soil microbial community. Most PLFA indicators are known to be
367 non-specific to a particular microbial group or even species of microorganism. Therefore,
368 future studies using 16S rDNA/18S rRNA analyses will be required to access specific
369 groups and identify microorganisms at the species level in order to advance our
370 understanding of the experimental factors.

371 **5. Conclusion**

372 Eighteen months after application, liming improved the soil chemical properties
373 (increasing the pH, increasing the exchangeable content of Ca and Mg, and decreasing
374 the exchangeable Al), which had a negative impact of liming on Gram+ bacteria and
375 actinomycetes in the soil surface. On the other hand, our study confirmed the hypothesis
376 that no-till ICLS conducted under system fertilization and liming favor the fungi soil
377 community. Interestingly, combining an integrated system or system fertilization with
378 lime favors the AMF community in the subsurface soil layer. In addition, sheep grazing
379 increased by 50% the community of saprophytic fungi in the subsurface soil layer
380 compared to the specialized system. We conclude that by benefiting the soil fungi
381 community in the subsurface soil layer, the association of liming, system fertilization, and
382 sheep grazing conducted in ICLS is considered key elements to improve the soil quality
383 in highly weathered agricultural soils.

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390 **Ethics approval**

391 This study follows the rules of the ethics committee on the use of animals of the Federal
392 University of Rio Grande do Sul.

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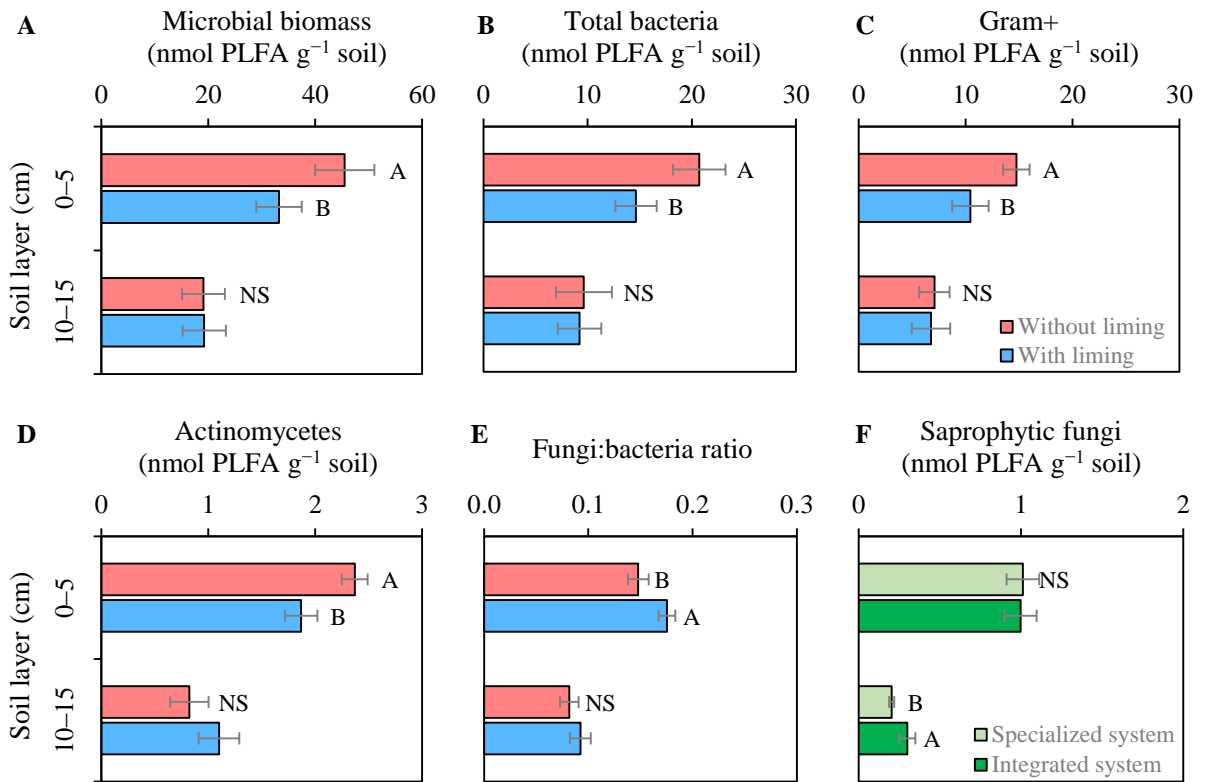


Figure 1. Microbial biomass (A), total bacteria (B), bacteria gram+ (C), actinomycetes (D), and fungi:bacteria ratio (E) as a function of liming, and saprophytic fungi (F) as a function of animal grazing in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Different letters differentiate the treatments within each layer by Tukey's test at a 5% significance level. NS = not significant.

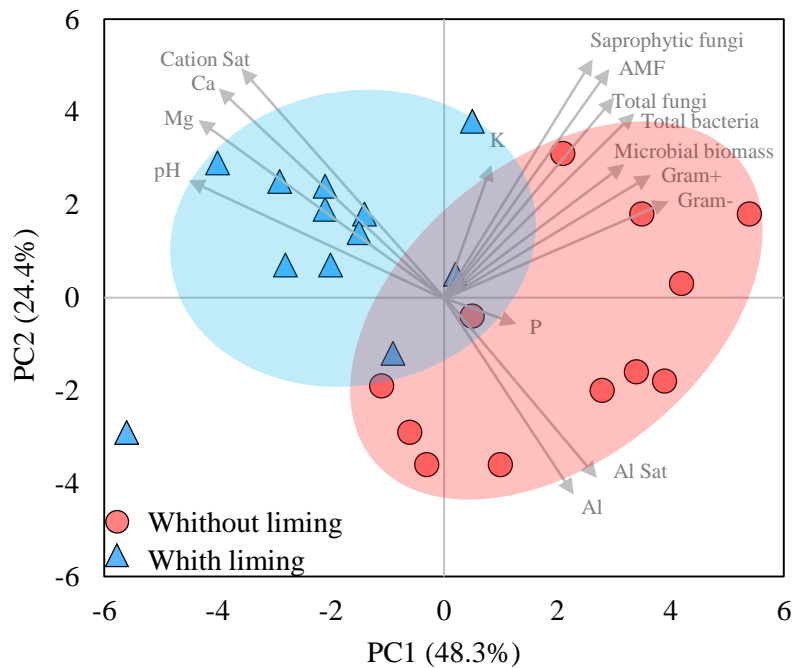


Figure 2. Scatter plot of principal component analysis (PCA) of microbial community by soil chemical properties at 0–5 cm soil layer in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil.

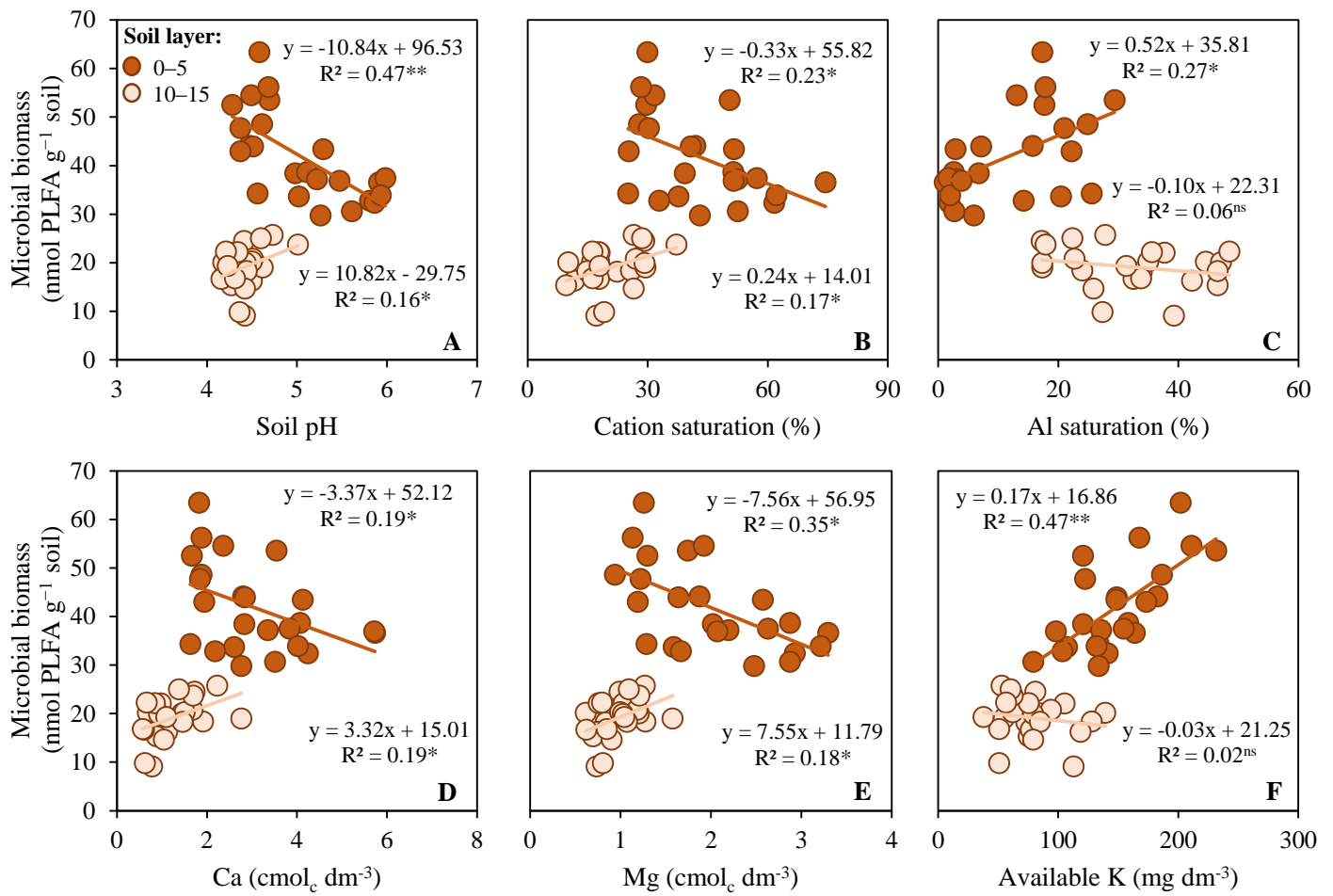


Figure 4. Relationships between microbial biomass relative to soil pH (A), cation saturation (B), Al saturation (C), exchangeable calcium (Ca) (D), exchangeable magnesium (Mg) (E), and available potassium (K) (F) in soil layers of 0–5 and 10–15 cm in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Regression were significant at * $p < 0.05$ and ** $p < 0.01$.

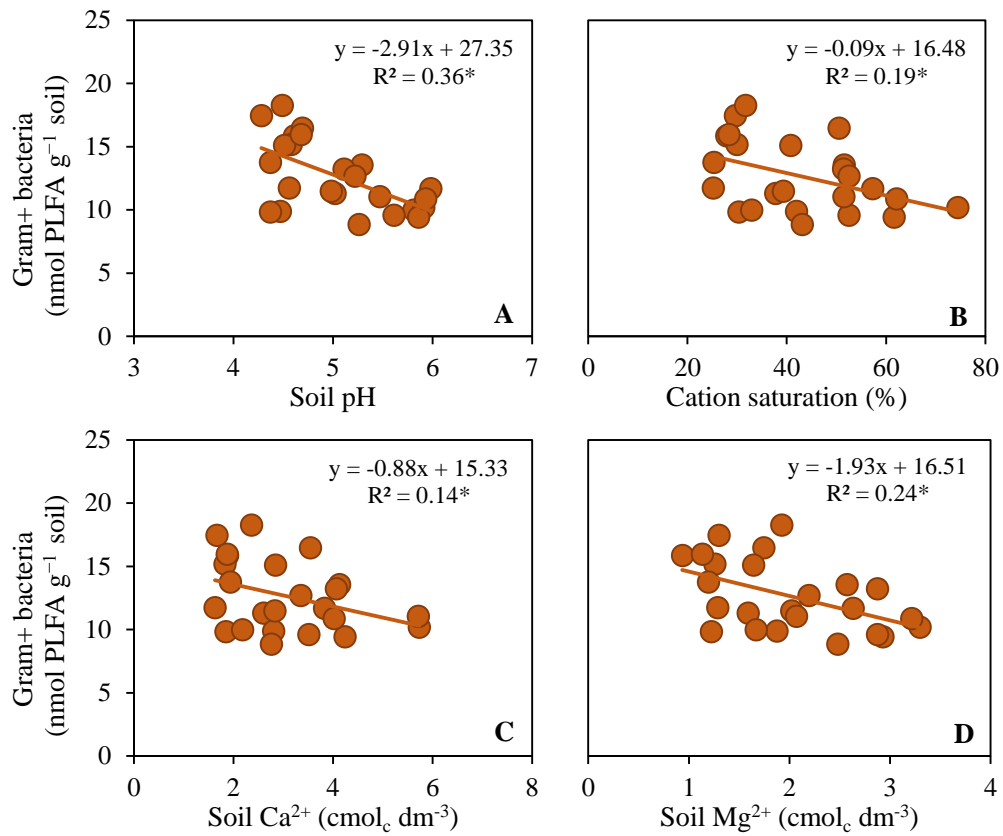


Figure 5. Relationships between gram+ bacteria to soil pH (A), cation saturation (B), exchangeable calcium (Ca²⁺) (C), and exchangeable magnesium (Mg²⁺) (D) in soil layer of 0–5 cm in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Regression were significant at *p< 0.05.

Table 1

Soil chemical and physical properties at the beginning of the field experiment in an Acrisol under no-tillage in Southern Brazil.

Soil layer cm	pH H ₂ O ^a	Available ^b		Exchangeable ^c			CEC _{pH7} ^d	Saturation ^e		TOC ^f	Particle-size distribution ^g		
		P	K	Ca	Mg	Al		Cation	Al		Clay	Silt	Sand
		mg dm ⁻³		cmol _c dm ⁻³				%			g kg ⁻¹		
0–10	3.9	94.2	76.6	1.1	0.5	1.6	12.4	14.9	48.5	1.7	134	239	627
10–20	4.0	45.8	68.5	0.9	0.4	1.6	11.8	14.5	53.1	0.8	149	234	618

^a Determined in water suspension (1:1, v/v).

^b Available P and K extracted by Mehlich-1.

^c Exchangeable Al³⁺, Ca²⁺, and Mg²⁺, extracted by 1.0 mol L⁻¹ KCl (1:10, v/v).

^d Cation exchange capacity at pH 7.0, calculated by summing H⁺, Al³⁺, Ca²⁺, Mg²⁺, and K⁺.

^e Cation saturation = (Ca²⁺ + Mg²⁺ + K⁺)/(CEC_{pH7}) × 100, and Al saturation = Al³⁺/(CEC_{effective}) × 100.

^f Total organic carbon was analyzed by dry combustion.

^g Particle-size distribution in clay <0.002 mm, silt 0.002–0.05 mm and sand >0.05 mm.

Table 2

Details of the pasture and cropping phases in the first three years of the experiment.

Pasture phase	Season	
	2017	2018
<i>Stocking period</i>		
Beginning	June 13	June 5
End	October 15	October 5
Grazing days	124	122
Italian ryegrass variety	BRS Ponteio	BRS Ponteio
Sowing rate (kg ha ⁻¹)	25	25
Nitrogen fertilization rate (kg ha ⁻¹)	150	150
Phosphorus fertilization rate (kg ha ⁻¹) ^a	30	30
Potassium fertilization rate (kg ha ⁻¹) ^a	58	58
<i>Animal information</i>		
Breed group	Corriedale	Corriedale
Age (months)	11	11
Initial weight (kg)	25	30
Stocking rate (kg ha ⁻¹ of live weight)	749	868
Cropping phase	Cropping period	
	2017/2018	2018/2019
Sowing	November 20	October 23
Harvest	April 27	April 23
Crop days	158	182
Soybean cultivar	DM 5958RSF IPRO	ND 5909
Sowing rate (seeds ha ⁻¹)	255 000	255 000
Phosphorus fertilization rate (kg ha ⁻¹) ^a	30	30
Potassium fertilization rate (kg ha ⁻¹) ^a	58	58

^a The amount of fertilizer (P and K) was applied either in the cropping phase (conventional fertilization) or in the pasture phase (system fertilization), as defined in the treatments.

Table 3

Fatty acids and their respective microbial groups.

Microbial group	Fatty acids
Gram+	i15:0; a15:0; i16:0; i17:0; a17:0
Gram-	cy19:0; cy17:0; 2-OH 10:0; 2-OH 12:0; 3-OH 12:0; 2-OH 14:0; 3-OH 14:0; 2-OH 16:0; 16:1□7
Actinomycetes	10-methyl 18:0; 10-methyl 19:0
Saprophytic fungi	18:2□9, 12
AMF ⁽¹⁾	16:1□5

⁽¹⁾Arbuscular mycorrhizal fungi.

Table 4

Significance of experimental factors and their interactions on changes in soil chemical properties for the 0-5 and 10-15 cm soil layers in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil, because analysis of variance (ANOVA).

Effect	Soil pH	Ca	Mg	Al	P	K	Al Saturation	Cation Saturation
----- <i>Soil layer of 0–5 cm</i> -----								
G ⁽¹⁾	0.8073	0.7355	0.6547	0.2265	0.1300	0.1633	0.5335	0.7147
F ⁽²⁾	0.4107	0.0906	0.6339	0.1111	0.2112	0.1167	0.2445	0.1111
L ⁽³⁾	0.0001**	0.0004**	0.0002**	0.0014**	0.6736	0.9415	0.0001**	0.0000**
G × F	0.9313	0.3831	0.01443	0.5399	0.4675	0.2015	0.9894	0.1112
G × L	0.2191	0.0750	0.0708	0.9541	0.7349	0.7243	0.4865	0.1414
F × L	0.9428	0.1305	0.2722	0.6876	0.4313	0.1804	0.9091	0.2051
G × F × L	0.1954	0.0781	0.1475	0.2777	0.3239	0.1630	0.1238	0.1909
CV (%)	5.5	24.1	13.7	26.3	25.7	19.5	13.6	14.9
----- <i>Soil layer of 10–15 cm</i> -----								
G	0.8237	0.9363	0.3457	0.7605	0.2283	0.6713	0.5465	0.8483
F	0.8844	0.9438	0.3335	0.4211	0.6412	0.6724	0.1081	0.3103
L	0.6091	0.0244*	0.2808	0.3819	0.9935	0.1164	0.4177	0.5907
G × F	0.2169	0.2258	0.9091	0.0833	0.1158	0.7156	0.7912	0.6931
G × L	0.3742	0.4761	0.4128	0.5926	0.2139	0.8051	0.4192	0.5012
F × L	0.3742	0.9887	0.3735	0.7277	0.3581	0.7377	0.5799	0.9046
G × F × L	0.0163	0.2998	0.8974	0.0974	0.4265	0.9876	0.1772	0.2214
CV (%)	4.7	34.1	31.5	25.2	31.6	32.7	28.0	29.2

⁽¹⁾Grazing. ⁽²⁾Fertilization strategy. ⁽³⁾Liming. *Significant $p < 0.05$. **Significant $p < 0.01$.

Table 5

Changes in soil chemical properties for soil layer 0–5 and 10–15 cm as a function of liming in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil.

Soil properties	0-5 cm		10-15 cm	
	Without liming	With liming	Without liming	With liming
Soil pH	4.5 ± 0.2 B	5.5 ± 0.4 A	4.4 ± 0.5	4.4 ± 0.2 ns
Exchangeable Ca (cmol _c dm ⁻³)	2.2 ± 0.6 B	3.9 ± 0.7 A	1.0 ± 0.1 B	1.5 ± 0.3 A
Exchangeable Mg (cmol _c dm ⁻³)	1.4 ± 0.3 B	2.6 ± 0.5 A	1.1 ± 0.4	1.1 ± 0.4 ns
Exchangeable Al (cmol _c dm ⁻³)	0.8 ± 0.3 A	0.2 ± 0.2 B	1.1 ± 0.5	1.0 ± 0.4 ns
Available P (mg dm ⁻³)	103.4 ± 19.6	108.2 ± 23.9 ns	52.0 ± 19.0	52.0 ± 26.0 ns
Available K (mg dm ⁻³)	162.2 ± 27.4	161.1 ± 24.4 ns	83.3 ± 14.0	64.6 ± 17.0 ns
Al saturation (%)	17.1 ± 6.9 A	4.0 ± 3.6 B	33.3 ± 11.2	30.3 ± 11.4 ns
Cation saturation (%)	33.2 ± 7.8 B	52.5 ± 11.0 A	21.6 ± 7.0	20.2 ± 7.4 ns

Different letters differentiate the treatments within each layer by Tukey's test at a 5% significance level. NS = not significant.

Table 6

Significance of experimental factors and their interactions on changes in soil microbial community for the 0-5 and 10-15 cm soil layers in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil, because analysis of variance (ANOVA).

Effect	POxC ⁽⁴⁾	Microbial biomass	Total bacteria	Gram+	Gram-	G+/G- ⁽⁵⁾	Actino. ⁽⁶⁾	AMF ⁽⁷⁾	Saprophytic fungi	Total fungi	Fungi/bacteria
----- 0-5 cm -----											
G ⁽¹⁾	0.6940	0.6876	0.3517	0.6257	0.2535	0.1934	0.2679	0.7727	0.4187	0.2457	0.3728
F ⁽²⁾	0.6638	0.4261	0.2567	0.3988	0.1407	0.2127	0.8562	0.5206	0.9931	0.5671	0.5425
L ⁽³⁾	0.1471	0.0097**	0.0054**	0.0040**	0.1054	0.1417	0.0428*	0.3598	0.4213	0.2895	0.0428*
G × F	0.1205	0.9895	0.8744	0.8869	0.2807	0.2278	0.1912	0.4000	0.8006	0.4367	0.4000
G × L	0.7123	0.3577	0.2195	0.4413	0.2050	0.2908	0.1186	0.5198	0.5948	0.5456	0.5198
F × L	0.5940	0.1882	0.1625	0.2435	0.1588	0.2098	0.6593	0.0904	0.4369	0.1245	0.0904
G × F × L	0.3873	0.4098	0.2779	0.3306	0.2816	0.2981	0.9827	0.5505	0.7166	0.4578	0.5505
CV (%)	19.8	25.6	26.6	24.4	40.3	34.4	28.6	28.4	54.5	34.5	25.4
----- 10-15 cm -----											
G	0.5096	0.2290	0.3193	0.1833	0.9066	0.7450	0.4882	0.1889	0.0418*	0.0988	0.0874
F	0.1308	0.3622	0.2161	0.4078	0.1002	0.2394	0.8222	0.5676	0.6356	0.5971	0.1935
L	0.3159	0.9608	0.8138	0.7664	0.6387	0.7123	0.1558	0.3243	0.8463	0.5460	0.2936
G × F	0.4155	0.2428	0.4324	0.6003	0.5274	0.4329	0.1273	0.0936	0.2491	0.1980	0.4563
G × L	0.4155	0.6273	0.4173	0.9088	0.1618	0.3456	0.3176	0.5068	0.6315	0.3013	0.1318
F × L	0.3633	0.2256	0.2084	0.2183	0.3547	0.2190	0.5473	0.2052	0.2339	0.2908	0.3958
G × F × L	0.2579	0.1408	0.4516	0.1353	0.3690	0.3412	0.1752	0.0352*	0.1065	0.1265	0.5866
CV (%)	33.5	39.3	43.4	38.2	35.9	31.3	47.1	52.4	55.1	49.9	32.69

⁽¹⁾Grazing. ⁽²⁾Fertilization strategy. ⁽³⁾Liming. ⁽⁴⁾Potassium permanganate-oxidizable C. ⁽⁵⁾Gram+:Gram- relations. ⁽⁶⁾Actinomycetes.

⁽⁷⁾Arbuscular mycorrhizal fungi. *Significant $p < 0.05$. **Significant $p < 0.01$.

Table 7

Changes in the arbuscular mycorrhizal fungi (AMF) community for soil layer 10–15 cm as a function of sheep grazing, fertilization strategy, and liming in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil.

Liming	Specialized system		Integrated system	
	Conventional fertilization	System fertilization	Conventional fertilization	System fertilization
	----- <i>nmol PLFA g⁻¹ soil</i> -----			
Without	0.5 ± 0.4 <i>Aaa</i>	0.5 ± 0.1 <i>Aaa</i>	0.7 ± 0.2 <i>Aaa</i>	0.5 ± 0.1 <i>Baa</i>
With	0.7 ± 0.3 <i>Aaa</i>	0.4 ± 0.2 <i>Aab</i>	0.4 ± 0.3 <i>Aba</i>	1.2 ± 0.5 <i>Aaa</i>

Capital letters compare the effect of liming within each animal grazing level and fertilization strategy. Lowercase letters compare the effect of fertilization strategy within each animal grazing level and liming. Lowercase letters in italics compare the effect of animal grazing within each level of fertilization strategy and liming.