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CALAGEM E ESTRATÉGIAS DE FERTILIZAÇÃO EM SISTEMAS AGROPECUÁRIOS: EFEITOS NA MICROBIOTA, DISPONIBILIDADE DE NUTRIENTES E PRODUTIVIDADE ANIMAL E VEGETAL

Lucas Aquino Alves

(Tese de Doutorado)

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TESE

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê.”

(Arthur Schopenhauer)

“Quando a moral se entrega o homem chega ao seu próprio fim, mas debaixo da macega se esconde o melhor do capim, debaixo do sombreiro tem um bugre missioneiro peleando dentro de mim.”

(Mano Lima)

Dedico a minha filha, Manuela.

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Gratidão a todos vocês!

CALAGEM E ESTRATÉGIAS DE FERTILIZAÇÃO EM SISTEMAS AGROPECUÁRIOS: EFEITOS NA MICROBIOTA, DISPONIBILIDADE DE NUTRIENTES E PRODUTIVIDADE ANIMAL E VEGETAL[1]

Autor: Lucas Aquino Alves
Orientador: Prof. Dr. Tales Tiecher

RESUMO

A inserção de animais em sistemas agrícolas combinada com a utilização correta da calagem e a escolha estratégica da época de aplicação de P e K podem promover melhoria na fertilidade do solo, na produtividade e na diversidade da microbiota do solo, resultando em um aumento significativo na eficiência produtiva e na sustentabilidade ambiental. Dessa forma, a presente tese tem como objetivo avaliar o efeito de diferentes estratégias de fertilização com P e K, do pastoreio ovino e da correção da acidez do solo pela calagem sobre a produção de plantas e animais, conteúdo de P e K no solo e tecido vegetal, e eficiência de uso e econômica da fertilização com P e K (*Capítulo II*); N fornecido através do processo fixação biológica de nitrogênio (FBN), ii) balanço parcial de N e produtividade da soja (*Capítulo III*); e a comunidade microbiana do solo e suas relações com os atributos químicos do solo (*Capítulo IV*). Em 2017, foi estabelecido uma experimento de campo em um Argissolo Vermelho distrófico cultivado com soja e azevém sob plantio direto. Nossos resultados demostraram que a fertilização de sistema e a calagem aumentaram o teor de P e K na biomassa do azevém. O uso do sistema integrado aumentou o retorno econômico e a eficiencia do uso do P e K . A estratégia de fertilização não afetou a produção de soja, mas aumentou a produção total de pastagem de azevém. Apesar disso, o peso vivo dos ovinos não aumentou. O montante de N fixado foi 27% maior no solo sob correção da acidez pela calagem. O balanço parcial de N foi positivo em ambos os tratamentos, mas 57% superior nos tratamentos com aplicação de calcário, resultando em 11% de aumento na produção de soja. Na camada superficial do solo, foi observado um impacto negativo da aplicação de calcário para as bactérias Gram+ e actinomicetos, impactando negativamente a comunidade bacteriana total e a biomassa microbiana. A combinação de um sistema integrado e fertilização de sistema com calagem aumentou a comunidade de fungos micorrízicos arbusculares na camada subsuperficial do solo. Além disso, o pastejo ovino aumentou a biomassa de fungos saprófitos em 50% na camada subsuperficial do solo. As propriedades químicas mais relevantes para o processo de FBN no solo foram o pH e a saturação por Al. As diferentes estratégias fertilização e o pastejo ovino não tiveram efeito sobre os atributos de acidez do solo, fornecimento de N e produtividade da soja. Além disso, o pastejo ovino e a estratégia de fertilização não afetaram as propriedades químicas do solo ou a comunidade microbiana na superfície do solo. No entanto, a calagem, a fertilização do sistema e o pastejo beneficiaram a comunidade fúngica do solo, melhorando assim a saúde de solos agrícolas altamente intemperizados.

Palavras-chave: acidez do solo, fertilização, fósforo, potássio.

[1]Tese de Doutorado em Ciência do Solo. Programa de Pós-Graduação em Ciência do Solo, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul. Porto Alegre. (84p.) Março, 2023.

LIMING AND FERTILIZATION STRATEGIES IN INTEGRATED SYSTEMS: EFFECTS ON MICROBIOTA, NUTRIENT AVAILABILITY, AND ANIMAL AND PLANT PRODUCTION[2]

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Adviser: Prof. Dr. Tales Tiecher

ABSTRACT

The insertion of animals in agricultural systems combined with the correct use of liming and the strategic choice of the timing of P and K application can promote improvement in soil fertility, productivity and soil microbiota diversity, resulting in a significant increase in productive efficiency and environmental sustainability. Thus, the present thesis aims to evaluate the effect of different P and K fertilization strategies, sheep grazing and soil acidity correction by liming on plant and animal production, P and K content in soil and plant tissue, and use and economic efficiency of P and K fertilization (*Chapter II*); N supplied through the biological nitrogen fixation (BNF) process, (ii) partial N balance and soybean yield (*Chapter III*); and the soil microbial community and its relationship to soil chemical properties (*Chapter IV*). In 2017, a field experiment was established on a Acrisol cultivated with soybean and ryegrass under no-till. Our results demonstrated that system fertilization and liming increased P and K content in ryegrass biomass. The use of the integrated systems increased the economic return and the use efficiency of P and K . The fertilization strategy did not affect soybean yield, but increased total production of Italian ryegrass pasture. Despite this, the live weight of the sheep did not increase. The amount of N-fixed was 27% higher in the soil with liming. The partial N balance was positive in both treatments, but 57% higher in the treatments with lime application, resulting in an 11% increase in soybean yield. In the surface soil layer, a negative impact of lime application was observed for Gram+ bacteria and actinomycetes, consequently decreasing the total bacterial community and total microbial biomass. The combination of an integrated system or system fertilization with lime increased arbuscular mycorrhizal fungi community 3.0-fold compared to a specialized system or conventional fertilization in the subsurface soil layer. In addition, sheep grazing increased saprophytic fungi biomass by 50% in the subsurface soil layer. The most relevant chemical properties for the BNF process in soil were pH and Al saturation. The different fertilization strategies and sheep grazing had no effect on soil acidity attributes, N supply and soybean productivity. In addition, sheep grazing and fertilization strategy did not affect soil chemical properties or the microbial community on the soil surface. However, liming, system fertilization, and grazing did benefit the soil fungal community, thus improving soil health in highly weathered agricultural soils.

Keywords: soil acidity, fertilization, phosphorus, potassium.

[2]Doctoral Thesis in Soil Science. Graduate Program in Soil Science, Faculty of Agronomy, Universidade Federal do Rio Grande do Sul. Porto Alegre. (84p.) March, 2023.

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CAPÍTULO I – Introdução geral

Solos tropicais e subtropicais, em grande parte, possuem sérias limitações a produção, no que se refere a acidez e disponibilidade de nutrientes. Dessa forma, práticas agronômicas de correção e fertilização tornam-se indispensáveis para a manutenção e aumento da produtividade dos cultivos. Além disso, cada vez mais busca-se sistemas de produção sustentáveis, que potencializem a produção do sistema do ponto de vista econômico, mas que tenham conexão com o ambiente. Nesse contexto, os sistemas integrados de produção agropecuária (SIPA) são reconhecidos pelas suas contribuições para a melhoria na produção agrícola, uma vez que intensificam de forma sustentável a produção de alimentos, além de promover melhorias na qualidade do solo.

Dessa forma, o objetivo geral da tese é ampliar o entendimento sobre os efeitos da correção da acidez do solo pela calagem e estratégias de fertilização com P e K em SIPA, avaliando os efeitos sobre a produção das culturas, nutrição de plantas, disponibilidade de nutrientes no solo, além dos efeitos sobre a microbiota. Serão apresentados na presente tese os resultados de três estudos, os mesmos foram desenvolvidos na Estação Experimental Agronômica da UFRGS (EEA-UFRGS), em um experimento que envolve a integração entre soja e ovinos de corte, correção da acidez do solo pela calagem e a adoção da estratégias de fertilização. Primeiramente avaliaremos o efeito dos tratamentos sobre a eficiencia do uso do fósforo e potássio no sistema *(1)*, eficiência da fixação biológica de nitrogênio e consequente disponibilidade de nitrogênio *(2)* e alterações na comunidade microbiana do solo *(3)*.

Acreditamos que nossos estudos trazem avanços e ampliam o conhecimento sobre a correção da acidez do solo em SIPA, além de balizar os novos conceitos de fertilização, baseado na estratégia de fertilização de sistema. Assim, nossas descobertas fornecerão uma base importante para orientar as estratégias de fertilização e correção da acidez do solo em SIPA, trazendo informações relevantes a comunidade cientifica e aos produtores rurais, os quais são os reais interessados pelos resultados obtidos.

CAPÍTULO II – Fertilization strategies and liming in no-till integrated crop–livestock systems: effects on phosphorus and potassium use efficiency[2]

1. Introduction

In weathered tropical and subtropical soils, phosphorus (P) and potassium (K) contents are typically low, resulting in a strong dependency on nonrenewable nutrient inputs (Stewart et al., 2005; SBCS, 2016; Dhillon et al., 2019). Despite being one of the world's largest food, fiber, and biofuel producers, Brazil is heavily dependent on fertilizer imports. In 2018 Brazil used approximately 35.5 million tons of fertilizers, 27.5 million (77 %) of which were imported, P mainly from Middle East countries and K from Canada (ANDA, 2019). For that reason, soil fertility management needs to be better planned to increase these nutrients' efficiency use.

Soil fertility management in tropical and subtropical areas, in general, aims to maintain adequate levels of available nutrients by building up soil P and K contents above thresholds values necessary for effective crop development (SBCS, 2016), which usually requires using large amounts of fertilizers. A subsequent fertilization process, which maintains the soil's nutrient status, usually requires less fertilizer input (usually the amounts needed to replenish nutrients removed for grain, fiber, or meat production in addition to losses by erosion, runoff and/or leaching) (SBCS, 2016; Pauletti & Motta, 2019).

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[2] Published in the Brazilian Journal of Soil Science (*Revista Brasileira de Ciência do Solo)* (Alves et al. 2022, doi: 10.36783/18069657rbcs20210125)

Increasing P and K use efficiency entails neutralizing soil acidity (Scanlan et al., 2017). An appropriate soil pH reduces P adsorption by Fe and Al oxides, and increases P availability to plants as a result, and also promote better root growth, increasing water use efficiency, and allowing plants to uptake nutrients from deeper soil layers, increasing nutrient efficiency (Gustafsson et al., 2012; Bai et al., 2017; Penn and Camberato, 2019; Alves et al., 2021; Bossolani et al., 2021). Amending soil acidity by liming also increases the cation exchange capacity (CEC) (Huang et al., 2020), mainly through deprotonation of surface functional groups present in organic matter —which is especially important in sandy soils to avoid K losses by leaching. Soil P and K availability are not only affected by acidity amendments, but also by factors like the animal component in integrated crop–livestock systems (ICLS).

Globally, ICLS is used to obtain more food per unit of land (Moraes et al., 2014), improve P and K cycling (Assmann et al., 2017), and increase the availability of these two nutrients in the soil (Ferreira et al., 2011; Deiss et al., 2016). Nevertheless, P and K efficiency can be further improved in ILCS, particularly if fertilizers are applied at the optimum time. Integrated crop–livestock systems typically alternates between a grain production phase with a higher nutrient exportation and a meat production phase with lower exportation. A long-term (14 years) study in Southern Brazil has shown that grain crops such as corn and soybean grown in the summer season may export up to 95 % of P and K from the soil, and sheep meat produced in winter pasture can export only 5 % (Alves et al., 2019). Thus, new fertilization strategies, which exploit nutrient cycling, transfers between organic and mineral phase, and increased soil biological activity in ICLS, are being envisioned with system fertilization (Ferreira et al., 2011; Deiss et al., 2016; Farias et al., 2020; Sekaran et al., 2021).

With conventional fertilization, P and K are supplied when the grain crops are sown as they require and export larger amounts of nutrients than the winter pasture (SBCS, 2016). Applying P and K to the soil at this time rapidly increase their availability and uptake by soybean plants. Following soybean harvest, the soil may return to a state of decreased availability of P and K, so the winter crop may not benefit from the fertilizer initially applied. By contrast, fertilization during the winter pasture phase can increase the soil’s available P and K levels throughout the growth period. As P and K exports through meat are minimal, it is expected that the nutrient availability in the soil will be sufficient for soybean production after the grazing period. Therefore, this system-focused strategy based on periods of high and low nutrient exports may increase P and K use efficiency.

Some studies have shown that soybean yields are unlikely to respond to fertilization in soils with high P and K availability (Boring et al., 2018). By contrast, anticipation fertilization can boost herbage production, especially if pasture is grazed (Farias et al., 2020). In fact, grazing in pastures with well-managed fertilization increases net aboveground primary production and root production (Souza et al., 2008; López-Mársico et al., 2015). Thus, because it boosts growth through multiple defoliation cycles, grazing can increase nutrient uptake from pasture relative to annual crops (Ruess et al., 1983; Chapin and McNaughton, 1989).

This study aimed to assess the effects of different P and K fertilization strategies, performed at different times: at summer soybean crop sowing – conventional fertilization, or at Italian ryegrass establishment in winter – system fertilization. These fertilization strategies were combined with grazing/ungrazed pasture and lime/control. Then, we evaluate the effect of these factors on herbage and animal production, soybean yield, soil available P and K budgets, plant nutrient uptake and use, and economic efficiency after three years, in a no-tilled Acrisol in a subtropical Brazilian region.

2. Material and methods

2.1. Experimental area and site

The field experiment started in April 2017, and it was set up at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA-UFRGS) in Eldorado do Sul, Rio Grande do Sul State, Brazil (30° 05’ S, 51° 39’ W, 46 m above sea level).

The climate in the region is classified as subtropical humid (Cfa) according to Köppen’s system (Alvares et al., 2013). Mean precipitation, air temperature and soil moisture during the experimental period (April 2017 to

March 2020), obtained from the EEA-UFRGS mobile automatic station (Weather Watch 2000, Campbell Scientific, Inc.), are presented in Fig. 1. The soil in the study site is Argissolo Vermelho distrófico (Santos et al., 2013), which corresponds to Acrisol (IUSS Working Group WRB, 2015); its main chemical and physical properties are summarized in Table 1.

The experimental area has been managed under no-tillage and ICLS since 2003. In the winter season (April to September), Italian ryegrass (*Lolium multiflorum* Lam.) pasture is grazed by sheep; the soil is cropped with soybean (*Glycine max*) in the summer (October to March).

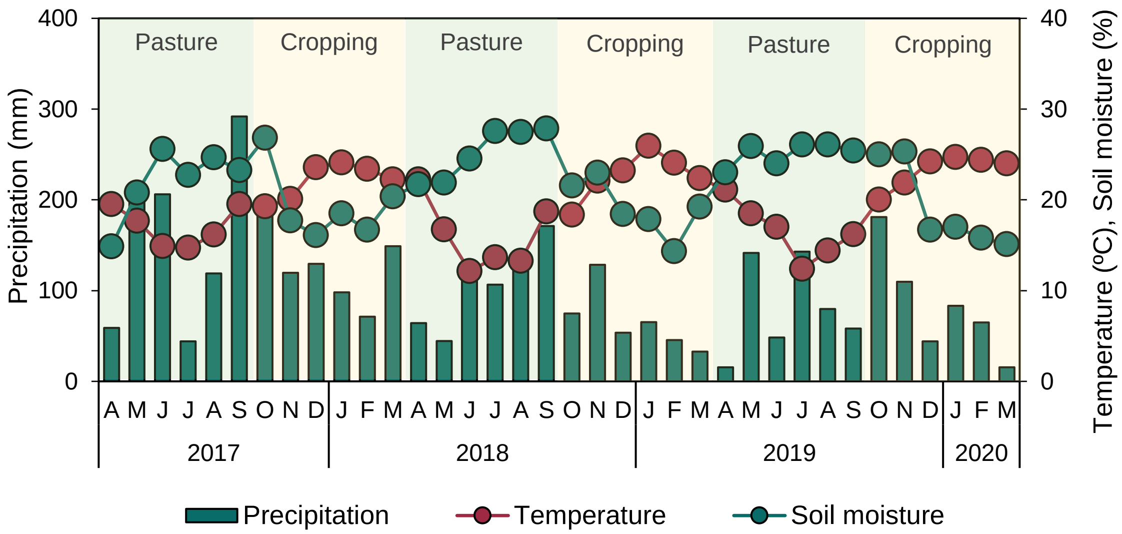

Figure 1. Precipitation, mean air temperature and soil moisture over the three pasture (2017, 2018, and 2019) and cropping cycles (2017/2018, 2018/2019, and 2019/2020) in the ICLS evaluated in Southern Brazil.

Table 1. Soil chemical and physical properties before the beginning of the field experiment (July 2017) in an Acrisol under no-tillage in Eldorado do Sul (Rio Grande do Sul State, southern Brazil).

Soil properties	Soil layer		Interpretation by SBCS (2016) for 0–10 cm soil layer	Reference
	0–10 cm	10–20 cm		
pH H_2O [a]	3.9	4.0	Below reference value	Soil pH below the reference value for no-tillage (pH ≥ 6.0)
Available P (mg dm^{-3}) [b]	94.2	45.8	Very high	Available P very high for a soil containing ≤ 200 g kg^{-1} of clay (30 mg dm^{-3})
Available K (mg dm^{-3}) [b]	96.6	68.5	High	Available K high for a $CECpH_{7.0}$ value of 7.6–15.0 $cmol_c$ dm^{-3} (90 mg dm^{-3})
Exchangeable Ca ($cmol_c$ dm^{-3}) [c]	1.1	0.9	Below reference value	Exchangeable Ca below the adequate threshold (4.0 $cmol_c$ dm^{-3}).
Exchangeable Mg ($cmol_c$ dm^{-3}) [c]	0.5	0.4	Below reference value	Exchangeable Mg below the adequate threshold (1.0 $cmol_c$ dm^{-3}).
Exchangeable Al ($cmol_c$ dm^{-3}) [c]	1.6	1.6	–	–
$CECpH_7$ ($cmol_c$ dm^{-3}) [d]	12.4	11.8	–	–
Cation saturation (%) [e]	14.9	14.5	Below reference value	Cation saturation below the reference value (≥ 65%)
Al saturation (%) [e]	48.5	53.1	Above reference value	Al saturation above the reference value (≤ 10%).
Soil organic carbon (g kg^{-1})	16.8	8.1	Medium	Medium soil organic carbon content (14.4–29.0 g kg^{-1})
Clay (g kg^{-1}) [f]	134	149	Class 4	Textural class 4 (≤ 200 g clay kg^{-1})
Silt (g kg^{-1}) [f]	239	234	–	–
Sand (g kg^{-1}) [f]	627	618	–	–

[a] In (1:1, v/v) water suspension. [b] Available P and K extracted by Mehlich-1. [c] Exchangeable Al^{3+}, Ca^{2+}, and Mg^{2+} extracted by 1.0 mol KCl L^{-1} (1:10, v/v). [d] Cation exchange capacity at pH 7.0 as calculated by combining H^+, Al^{3+}, Ca^{2+}, Mg^{2+} and K^+. [e] Cation saturation = (Ca^{2+} + Mg^{2+} + K^+)/(CEC_{pH7}) × 100, and Al saturation = Al^{3+}/($CEC_{effective.}$) × 100. [f] Particle size distribution in clay < 0.002 mm, silt 0.002–0.05 mm and sand > 0.05 mm.

2.2. Experimental design and soil management history

The experiment was established in 4.8 ha, split into 16 paddocks 0.23 0.41 ha in size. The experimental design was a completely randomized block with 4 replications in a 2 × 2 factorial system and split-plots. The first factor was animal grazing, that is, whether the land was grazed (integrated system) or ungrazed (specialized system). The second factor was P and K fertilization strategy, which involved supplying the soil with fertilizer in the soybean cropping phase (conventional fertilization) or winter pasture (Italian ryegrass) phase (system fertilization). The split-plot was the effect of acidity amendment (i.e., whether or not the soil was limed).

The effect of acidity neutralization (i.e., liming or no liming) was examined in all plots by excluding an area of 32 m^2 (4 × 8 m) from limestone application to maintain the original acidity conditions (subplot). Table 1 summarizes the chemical properties of the soil at that point. Soil acidity was amended by applying limestone in the amount needed to raise the pH (H_2O) to 6.0, as recommended by the local Soil Fertility Committee (SBCS, 2016). The soil was limed with 7.5 Mg ha^{-1} of dolomitic limestone [$CaMg(CO_3)_2$] with an effective neutralizing power of 72 %. Liming was performed in July 2017 on the soil surface without incorporation.

Grazing started in June or July of 2017, 2018, and 2019, and finished in October each year (Table 2). Italian ryegrass was established by sowing viable seeds at a rate of 25 kg ha^{-1} with a centrifugal distributor in May of each year. Urea (45 % N) was applied at a rate of 150 kg ha^{-1} of N to all paddocks at the ryegrass V3 stage (3 totally expanded leaves). All treatments received N fertilization. Table 2 shows detailed information about the sheep. The stocking rate was adjusted by put-and-take method (Mott and Lucas, 1952) and it was used to maintain the average sward canopy height (SCH) at 0.15 m, which provides the optimum plant structure for maximizing animal production (Carvalho, 2013). The SCH was monitored at 7-day intervals using a sward stick (Barthram, 1985) to measure 150 randomly chosen points per experimental unit monthly. Immediately, at the end of the pasture phase, residual ryegrass was desiccated with glyphosate herbicide before soybean was sown.

The P and K fertilization rates were calculated from the amounts of P and K removed by soybean grains at an expected yield of 4.0 Mg ha^{-1}. In the soybean crops of 2017/2018 and 2018/2019 were applied 30 kg ha^{-1} of P and 58 kg ha^{-1} of K, and in 2019/2020 crop season a rate of 25 kg ha^{-1} of P and 67 kg ha^{-1} of K was used (Table 2). Fertilization rates were calculated according to SBCS (2016). The application of P and K fertilizer was performed on the soil surface, both in the conventional fertilization treatment, coupled with soybean sowing, and in the system fertilization, at the establishment of the Italian ryegrass. As can be seen in Table 1, soil available P and K levels at the beginning of the experiment exceeded the critical thresholds. Table 2 shows the cultivars, sowing method, harvesting date, and sowing density for the 2017/2018, 2018/2019, and 2019/2020 seasons. Soybean was sown in rows 45 cm apart in all cropping seasons.

2.3. Assessment of soil acidity, soil available P and K, and P and K contents in plant tissue

Phosphorus and K content in soil and plant were determined at four different sampling times from 2017 to 2019. In the winter pasture phase, soil and plant samples were collected after 100 days of grazing to determine soil pH, and Ca^{2+}, Mg^{2+}, and Al^{3+} in September 2017 and 2018. In the cropping phase (January), samples were collected at the R1 soybean stage in the 2017/2018 and 2018/2019 cropping seasons (Fehr & Caviness, 1977). The soil was sampled with an auger in the 0.00-0.20 m layer. Samples were dried in a forced-air oven at 45 °C, the larger lumps being crumbled, ground, and sieved (Ø = 2.0 mm). Soil pH was measured in aqueous suspensions (1:1 ratio, v/v). Available P and K were extracted with Mehlich-1, and Al^{3+}, Ca^{2+}, and Mg^{2+} with KCl 1.0 mol L^{-1} (Tedesco et al., 1995). Cation and Al saturation were calculated according to SBCS (2016).

Italian ryegrass tissue was sampled after 100 days of grazing, six sub-samples from each paddock being combined into a composite sample. Each sample was obtained by clipping at ground level the blades inside a 0.25 m^2 (0.5 × 0.5 m) quadrat. Soybean tissue was obtained at the R1 stage by cutting whole plants near ground level. Four sub-samples per paddock of 2 linear meters (2.0 × 0.45 m) were combined to obtain a composite sample. Both pasture and soybean samples were dried in a forced-air oven at 65 °C, weighed on an analytical balance, milled, and sieved (Ø = 0.5 mm). The P and K contents of plant tissue were determined after chemical digestion with H_2O_2 + H_2SO_4 according to Tedesco et al. (1995).

Table 2. Details of the pasture and cropping phases in the three years of the experiment.

Pasture phase	Season		
	2017	2018	2019
Stocking period			
Start of stocking period	June 13	June 5	July 5
End of stocking period	October 15	October 5	October 26
Grazing days	124	122	113
Italian ryegrass variety	BRS Ponteio	BRS Ponteio	BRS Ponteio
Sowing rate (kg ha^{-1})	25	25	25
Nitrogen fertilization (kg ha^{-1})	150	150	150
Animal information			
Breed group	Corriedale	Corriedale	Corriedale
Age (months)	11	11	10
Initial live weight (kg)	25	30	31
Cropping phase	Cropping period		
	2017/2018	2018/2019	2019/2020
Sowing	November 20	October 23	November 28
Harvest	April 27	April 23	April 13
Crop days	158	182	137
Soybean cultivar	DM 5958RSF IPRO	ND 5909	TMG 7063 IPRO
Sowing rate (seeds ha^{-1})	255 000	255 000	255 000
Phosphorus fertilization (kg ha^{-1})	30	30	25
Potassium fertilization (kg ha^{-1})	58	58	67

2.4. Soybean, pasture, and animal production

Soybean yield, total herbage production, and live weight gain (LWG) per hectare were evaluated over three seasons. Soybean yield (kg ha^{-1}) was determined in five random sub-samples (2.0 × 0.45 m) from each plot (total area 4.5 m^2). Samples were collected at physiological maturity, threshed to determine grain moisture, and yield was calculated adjusting the moisture level to 130 g kg^{-1}.

Total herbage production as dry matter (kg DM ha^{-1}) was calculated as the daily herbage accumulation rate (kg DM day^{-1}) for each stocking period multiplied by the number of days of the period and that of stocking periods, the result being added to the initial herbage mass as determined one day before the start of the stocking period. Residual herbage mass at the end of the stocking cycle was estimated identically with herbage mass. The daily herbage accumulation rate was determined by using 4 grazing exclusion cages per experimental unit, the herbage mass inside each cage being clipped at ground level at 28-day intervals. On the other hand, the daily herbage accumulation rate was obtained as the difference between herbage mass in the grazing exclusion cage and pasture mass at the beginning of each stocking period divided by the number of days of the period.

Animals were weighed at the beginning and end of each grazing period (28 ± 3 days) to adjust stocking rates and monitor animal performance. The total LWG per hectare (kg ha^{-1}) was calculated as the difference between the final and initial weight of tester sheep multiplied by the number of animals per hectare and divided by the paddock area (ha).

2.5. Soybean, pasture, and animal production

All P and K inputs via fertilizer and outputs through soybean grains and sheep meat were considered in the total budget. Phosphorus and K removal by soybean grains were calculated using the mean values adopted by SBCS (2016), namely: 6.1 kg P Mg^{-1} of grain and 16.6 kg K Mg^{-1} of grain. The nutrient contents of sheep meat (LWG) were calculated according to Williams

(2007) (i.e., on the assumption that the sheep removed 0.194 g kg^{-1} of P and 0.344 g kg^{-1} of K).

Soil available P and K budgets were calculated with provision for the initial and final available P and K contents in the 0.00-0.20 m soil layer, and all inputs (fertilizer) and outputs (LWG and grain biomass) (Alves et al., 2019):

$$SB = (FS - IS) - (IF - OGM) \quad \textbf{Eq. 1}$$

in which: SB denotes soil budget; FS denotes final soil content (January 2019); IS denotes initial soil content (July 2017); IF means inputs via fertilizer; and OGM means outputs via grain and meat. All units have been converted to kg ha^{-1}.

2.6. Economic and use efficiency of P and K fertilization

Use efficiency (UE) for protein production per P and K fertilizer unit applied was calculated as 5.71 times (Merrill and Watt, 1973) the N content of soybean (SBCS, 2016). For live weight gain, carcass yield in Corriedale lambs was assumed to be 44.1 % (Carvalho et al., 2006) and protein content 20.4 % (Kremer et al., 2004). The P and K use efficiency were calculated according to equation 2.

$$UE = Prot_{total}/Nutr_{applied} \quad \textbf{Eq. 2}$$

in which: Prottotal denotes the total amount of protein produced in soybean grains and sheep live weight over 3 years, and Nutraplied denotes the total amount of nutrient (P or K) applied via fertilizer in the same period.

Economic efficiency (EE) per fertilizer P and K unit applied was calculated from the economic return of soybean and meat production of sheep in US dollars (USD), using the average price for the previous three years (CEPEA, 2020):

$EE = USD_{total}/Nutr_{applied}$ **Eq. 3**

in which: USDtotal is the total economic return from soybean grains and sheep LWG for the three-year period; and Nutraplied is the total amount of nutrient supplied via fertilizer in the same period.

2.7. Statistical analyses

Statistical analyses were performed with the software SAS® 9.4 (SAS, 2015). The results were checked for normality with the Shapiro–Wilk test and variance homoscedasticity with the Levene test, both at a significance level of 5 %, prior to analysis of variance (ANOVA, $p<0.05$). When significant, differences between treatment means were evaluated with Tukey’s test, also at the 5 % significance level.

The effects included in the statistical model were fertilization strategy (conventional or system fertilization), grazing (specialized or integrated system), and liming (with or without). Fertilization strategy (F), grazing (G), liming (L), and the interactions F*G, F*L, G*L and F*G*L, were used as fixed effects, and block and its interactions as random effects. We use the PROC MIXED procedure and RANDON effect in SAS® 9.4 (SAS, 2015). The models for available P and K in soil, total herbage production, LWG per area and soybean yield included the fixed effect of year (Y) and its interactions with other factors.

3. Resultados

3.1. Soil acidity, and P and K contents of soil and plants

Soil acidity was affected by neither grazing nor fertilization (Table 3). Liming increased soil pH (4.3 to 5.0), Ca^{2+} (1.7 to 2.4 $cmol_c$ dm^{-3}), Mg^{2+} (1.2 to 1.9 $cmol_c$ dm^{-3}) and cation saturation (26.1 to 37.3 %), and decreased Al^{3+} (1.0 to 0.7 $cmol_c$ dm^{-3}) and Al saturation (25.7 to 17.3 %), in the 0.00-0.20 m soil layer after 18 months (Table 3).

Table 3. Acidity-related chemical properties of the soil in the 0–20 cm layer 18 months after limestone application in the ICLS.

Soil property	Without liming	With liming
pH	4.3 ± 0.3 B	5.0 ± 0.3 A
Cation saturation (%)	26.1 ± 4.2 B	37.3 ± 5.4 A
Al saturation (%)	25.7 ± 3.9 A	17.3 ± 2.8 B
Exchangeable Ca ($cmol_c$ dm^{-3})	1.7 ± 0.4 B	2.4 ± 0.5 A
Exchangeable Mg ($cmol_c$ dm^{-3})	1.2 ± 0.2 B	1.9 ± 0.2 A
Exchangeable Al ($cmol_c$ dm^{-3})	1.0 ± 0.1 A	0.7 ± 0.1 B

Different letters in each row denote significant differences as per Tukey’s test ($p < 0.05$).

Available P and K in the 0.00-0.20 m soil layer was affected by neither grazing nor fertilization strategy at any time during the sampling period (Fig. 2a). Soil available P was lower in the cropping phase of the 2018/2019 season (53 mg dm^{-3}) than it was in the pasture phases of 2017 (91 mg dm^{-3}) and 2018 (83 mg dm^{-3}), and in the cropping phase of 2017/2018 (90 mg dm^{-3}) (Fig. 2a). Liming had no effect on soil available P (mean of 77 mg dm^{-3}; Fig. 2b). Available K was lower in the 2017/2018 cropping phase (76 mg dm^{-3}) than it was in the 2017 and 2018 pasture phases (111 and 122 mg dm^{-3}, respectively), and in the 2017/2018 cropping phase (100 mg dm^{-3}) (Fig. 2c). Also, it was lower with liming (102 mg dm^{-3}) than without it (90 mg dm^{-3}) (Fig. 3c).

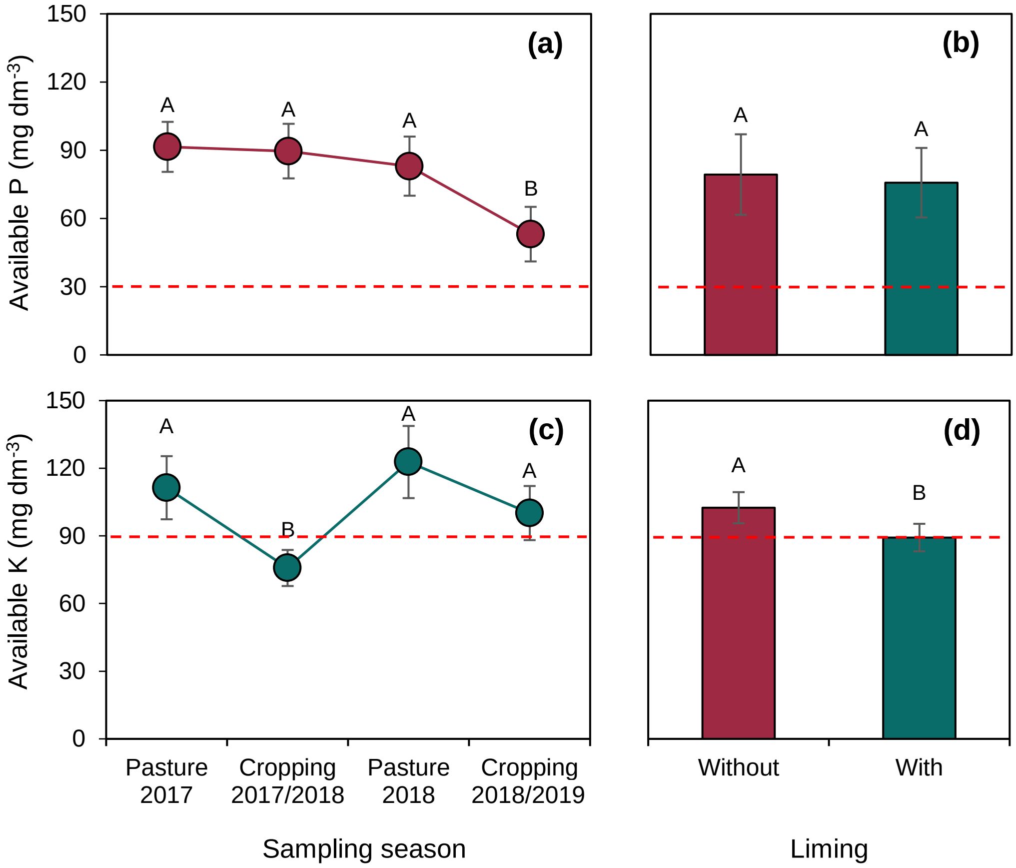

Figure 2. Available phosphorus (P) in the 0–20 cm soil layer as affected by sampling season (**a**) and liming (**b**), and available potassium (K) in the soil as affected by sampling season (**c**) and liming (**d**) (with or without liming). The red dotted lines represent the reference values for available P (30 mg dm^{-3}) and available K (90 mg dm^{-3}) in soil containing ≤ 200 g dm^{-3} clay and having a CECpH7 value of 7.6–15 $cmol_c$ dm^{-3} (SBCS, 2016). Different letters denote significant differences as per Tukey's test ($p < 0.05$).

Phosphorus and K contents of aboveground pasture biomass were affected by grazing, fertilization strategy and liming (Fig. 3). Thus, P contents in the 2017 and 2018 pasture seasons were higher with system fertilization than with conventional fertilization (3.4 vs 2.6 g kg^{-1} in 2017 and 5.8 vs 4.2 g kg^{-1} in 2018; Fig. 3a). Similar results were obtained as regards grazing. Thus, P contents were higher with the integrated system than they were with the specialized system in both pasture seasons (viz., 3.3 vs 2.6 g kg^{-1} in 2017 and 5.6 vs 4.6 g kg^{-1} in 2018; Fig. 3c). Potassium contents were higher with system fertilization than with conventional fertilization (viz., 16.5 vs 19.7 g kg^{-1} in 2017 and 31.5 vs 24.9 g kg^{-1} in 2018; Fig. 3b); also, they were higher with the integrated system than with specialized system (20.3 vs 15.3 g kg^{-1} in 2017 and 32.3 vs 24.1 g kg^{-1} in 2018; Fig. 3d). Finally, P contents in the 2018 pasture season were higher with liming than without it (5.1 vs 4.6 g kg^{-1}; Fig. 3e), and so were K contents (29.4 vs 26.9 g kg^{-1}; Fig. 3f).

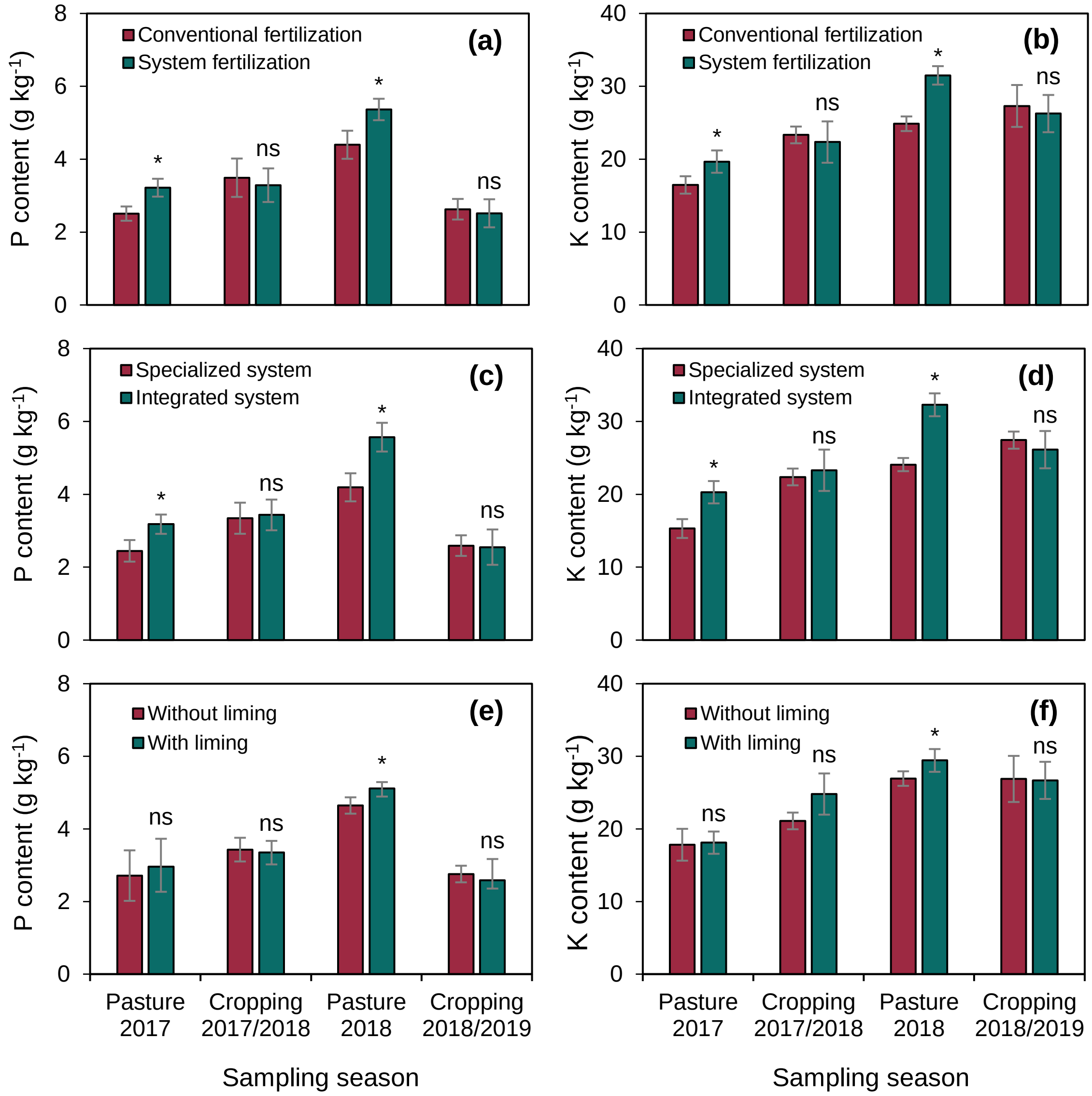

Figure 3. Phosphorus (P) and potassium (K) contents in aboveground biomass as affected by fertilization strategy (conventional or system) (**a, b**), animal grazing (specialized or integrated) (**c, d**), and liming (with or without) (**e, f**). Different letters denote significant differences as per Tukey's test ($p < 0.05$).

3.2. Soybean, pasture, and animal production

Average production of Italian ryegrass herbage in the studied period (2017–2019) was higher with the integrated system than with the specialized system (8616 vs 7795 kg DM ha^{-1}; Fig. 4a). Ryegrass production was also greater with system fertilization than conventional fertilization (8879 vs 7657 kg DM ha^{-1}; Fig. 4a). Even so, the sheep LWG per unit area was similar to both fertilization strategies. Also, LWG was higher in the pasture phases of 2017 (300 kg ha^{-1}) and 2018 (325 kg ha^{-1}) than it was in 2019 (213 kg ha^{-1}) (Fig. 5a).

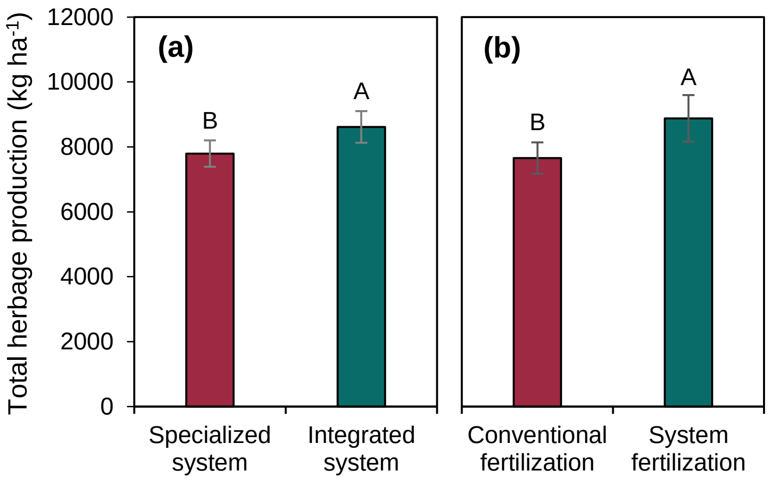

Figure 4. Total herbage production of Italian ryegrass (average of the years 2017, 2018 and 2019) as affected by animal grazing (specialized or integrated) (**a**) and fertilization strategy (conventional or system) (**b**). Different letters denote significant differences as per Tukey's test ($p < 0.05$).

Soybean yield exhibited no substantial differences between grazing or fertilization strategies (Fig. 5b). Moreover, it decreased over time, from 2.90 Mg ha^{-1} in the 2017/2018 season to 2.64 Mg ha^{-1} in 2018/2019 and 2.51 Mg ha^{-1} in 2019/2020 (Fig. 5a). Also, the average soybean yield for the three cropping seasons was higher with liming than without it (2.77 vs 2.59 Mg ha^{-1}; Fig. 5c).

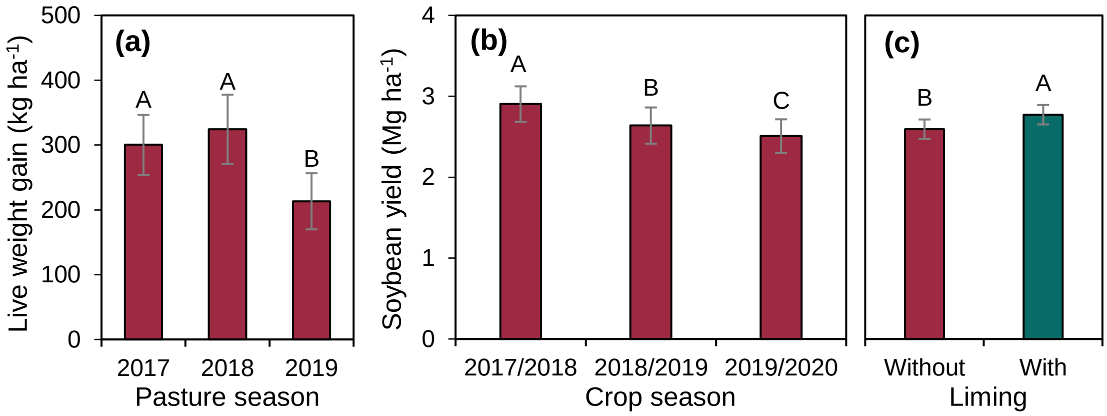

Figure 5. Live weight gain of sheep per unit area in each pasture season (**a**), and soybean yield as affected by crop season (**b**) and liming (with or without) (**c**). Different letters denote significant differences as per Tukey's test ($p < 0.05$).

3.3. Soil P and K budgets

Available P budget in soil was positive and affected by none of the treatments. On the other hand, the K budget was negative with all treatments, but higher with the system fertilization-integrated system combination (-25.6 kg K ha^{-1}) than it was with the conventional fertilization-specialized system, conventional fertilization–integrated system and system fertilization-specialized system combinations (-68.8, -95.3 and -108.9 kg K ha^{-1}, respectively; Table 4). Liming, however, had no effect on the available P and K budgets.

Table 4. Available P and K budgets in the 0–20 cm soil layer as affected by fertilization strategy (conventional or system fertilization) and animal effect (specialized system or integrated system) in the ICLS.

Fertilization	Grazing	Soil budget (kg ha^{-1}) [a]	
		Stock of available P	Stock of available K
Conventional	Specialized system	49.9 ± 10.8	–68.8 ± 13.2 B
	Integrated system	30.3 ± 8.6	–95.3 ± 12.1 BC
System	Specialized system	26.3 ± 7.8	–108.9 ± 19.1 C
	Integrated system	36.9 ± 9.4	–25.6 ± 8.2 A
Mean		35.8 [ns]	–74.6 *

[a] Calculated as SB = (FS – IS) – (IF – OGM), where SB = soil budget, FS = final soil content, IF = inputs via fertilizer, IS = initial soil content and OGM = outputs via grain and meat. [ns] Not significant at $p < 0.05$. *Significant at $p < 0.05$. Different letters distinguish the soil K budget under the effect of the interaction between fertilization strategy (conventional or system fertilization) and grazing (specialized system or ICLS) as per Tukey's test ($p < 0.05$).

3.4. Economic and use efficiency of P and K fertilization

Increased protein production per P and K unit applied via fertilizer resulted in an increased use efficiency with integrated system (35.1 kg P kg^{-1} and 16.4 kg K kg^{-1}) relative to specialized system (29.7 kg P kg^{-1} and 13.9 kg K kg^{-1}) (Fig. 6a). Also, the increased economic return, in dollars, from soybean production and sheep LWG per P and K unit applied via fertilizer resulted in increased economic efficiency with the integrated system (49.7 USD kg^{-1} P and 23.3 USD kg^{-1} K) relative to the specialized system (30.9 USD kg^{-1} P and 14.5 USD kg^{-1}) (Fig. 6b).

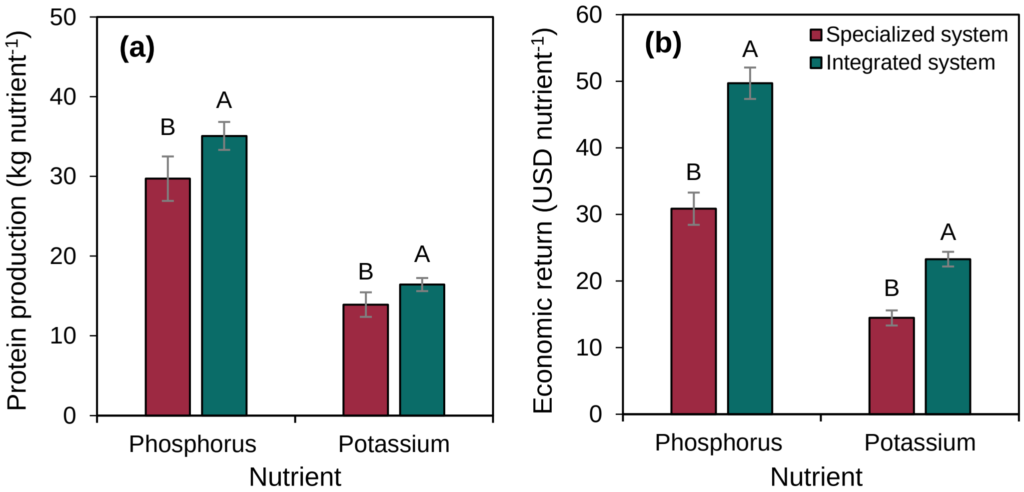

Figure 6. Phosphorus and potassium use efficiency for protein production (**a**) and economic return (**b**) as affected by grazing. Different letters denote significant differences as per Tukey's test ($p < 0.05$).

4. Discussion

4.1. Effect of soil acidity amendment by liming

Liming has well-known benefits on plant nutrition, like greater nutrients absorption such as N, P, K, Ca, Mg, and S, which leads to higher crop yields (Goulding, 2016; Holland et al., 2018, 2019; Bossolani et al., 2021). In this study, it increased the P and K contents of Italian ryegrass aboveground biomass in the 2018 pasture season (Fig. 3a and 3b). Amending soil acidity by liming boosts root development and shoot growth (Fageria and Nascente, 2014), and increased root growth facilitates the exploration of deeper soil layers, facilitating P and K uptake by plants.

Although the applied lime rate was calculated to raise the pH to 6.0, the soil pH in the 0.00-0.20 m layer only reached 5.0 after 18 months. The limited effect of surface liming in the first few centimeters of soil is recurrent in no-tillage areas (Rheinheimer et al., 2018; Miotto et al., 2019), and proportional to the reaction time after application. Therefore, this result is explained by the short-term effect combined with the low solubility of lime. Moreover, the faster reactivity of the finer particles in the first centimeters raises the soil pH and Ca^{2+} content, resulting in a slow dissolution of the coarser particles of lime, hampering soil acidity correction (Scott et al., 1992). For these reasons, it is recommended that in areas with chemical restriction in-depth (>20-30 % Al saturation) associated with available P content below the critical level, liming with incorporation should be always carried out (SBCS, 2016). As the initial levels of available P in the soil were above the critical level in the experimental area, liming was carried out on the soil surface. Despite not having increased the soil pH to the target value in the 0.00-0.20 m layer (pH 6.0), and although in our study Al^{3+} was not completely neutralized by liming this soil layer, it is still possible to verify the beneficial effect of liming on soybean productivity (Fig. 5c) (Holland et al., 2019).

Despite that soybean yield was higher in the liming treatment, the average yield of the treatments in the three harvests (2.7 Mg ha^{-1}) was below the expected 4 Mg ha^{-1} used to calculate the required P and K rates. Therefore, the residual acidity (pH values below 5.5 and Al saturation >10 %) in the liming treatment may be the limiting factor to achieve the target soybean yield (SBCS, 2016). In addition, another factor that has been limiting crop yields in the experimental area is water restriction, as droughts are frequently documented (Alves et al., 2020). Some studies under ICLS previously showed that liming does not affect soybean yield in moderately acidic soils (pH 4.8, cation saturation 56 % and Al saturation 15 % in the 0.00-0.20 m deep layer) (Martins et al., 2014a). These conditions, however, are contrasting from those of our soil, which had an initial pH of 4.0, cation saturation of 15 %, and Al saturation of 50 % in the 0.00-0.20 m layer, all of which led to an increased soybean yield.

4.2. Effect of sheep grazing and fertilization strategy

Higher herbage production obtained with the system fertilization results from the higher P and K contents in the aboveground biomass of Italian ryegrass (Fig. 3a and 3d). By contrast, P and K content in soybean biomass did not differ between fertilization strategies, resulting in similar grain yields. This result testifies to the high potential of system fertilization for improving nutrient availability and plant nutrition, largely due to P and K fertilizer being applied in the pasture phase. The fact that P and K supply was maintained throughout the pasture phase was a result of the heavy cycling of nutrients caused by grazing and by the sheep returning most of the nutrients ingested through dung and urine (Ferreira et al., 2011; Silva et al., 2014; Deiss et al., 2016; Assmann et al., 2017; Alves et al., 2019).

Increased total production of Italian ryegrass is consistent with the increased herbage production previously found by Farias et al. (2020). Nitrogen, P, and K fertilization usually increase total herbage production by providing a greater supply of fodder to sheep, thereby increasing meat production (Ihtisham et al., 2018). Although the increase in total herbage production resulted in no substantial increase in LWG per area here (Fig. 5a), gains are expected to become apparent in the long term. However, using pasture residues as inputs is crucial to regulate the soil C stock to maintain soil fertility and nutrient availability and have a positive effect on soybean yield in the long term (Alves et al., 2020).

The fact that soybean yield failed to respond to earlier P and K fertilization may have resulted from the curve of nutrient uptake by grain crops comprising a single cycle. In addition, the soil P and K content were above the critical level, thus the likelihood of crop response is much lower (Oliveira Junior et al., 2016; SBCS, 2016). This result is interesting as it is one of the first field studies demonstrating that system fertilization can be an effective strategy to enhance system production. Unlike grain crops, pasture is continuously stimulated to grow and uptake nutrients from the soil due to defoliation (Gastal and Lemaire, 2015). Grazing can increase photosynthetic and growth rates in plants (Gifford and Marshall, 1973), thereby increasing the requirements for nutrient uptake over several cycles of growth stimulation during grazing (Ruess et al., 1983; Chapin and McNaughton, 1989). This is consistent with the increased ryegrass herbage production observed when P and K were supplied in the pasture phase. This result, however, should only be expected when soil P and K levels exceed a critical threshold (Table 1). Our results cannot be extrapolated to soil conditions where P and K levels are below the critical level. Thus, further studies should be conducted using soils with available P and K contents below the critical level, as well as observing if other nutrients, such as N, could be managed in system fertilization. We emphasize that for a good functioning of the production system and for the system fertilization to be efficient, it is necessary to take into account some prerequisites, such as proper fertilization, soil acidity neutralization, use of the no-till system, and the adoption of ICLS (Anghinoni and Vezzani, 2021).

Once the amount of K exported by grains and meat was lower than the K inputs via fertilizers, the negative soil available K budget obtained with all treatments was possibly a result of excess K accumulating in non-exchangeable and structural forms in the soil - a frequent occurrence in soils containing 2:1 clay minerals (Watson et al., 2002; Berry et al., 2003) as found in previous studies in the same experimental area (Alves et al., 2019). In fact, 2:1 clay minerals can easily fix K in their interlayer spaces (Ernani et al., 2007). Also, applied K can partly migrate to soil layers below 0.20 m. In any case, the soil K budget was 2.7 times greater with the system fertilization–integrated system combination than with the conventional fertilization–specialized system combination. This result testifies the importance of animal grazing when nutrients are applied via fertilizer at an earlier time. In fact, grazing boosts root production (López-Ársico et al., 2015), thereby expanding nutrient uptake volume. In addition, continuous growth stimulation by grazing increases the requirements for nutrient uptake (Ruess et al., 1983; Chapin and McNaughton, 1989), thus hindering potential losses of K through leaching and runoff. By contrast, the soil available P budget was positive and similar to all treatments (Table 3). This was largely the result of the fertilization history in the experimental field (Alves et al., 2019), leading to saturation of the most P-eager sites and reducing the ability of the soil to immobilize P, converting it into less available forms as a result.

Integrated system provides greater economic returns than a specialized system (Franzluebbers, 2007; Sulc and Tracy, 2007; Oliveira et al., 2014). The increased protein production (use efficiency) and economic return (economic efficiency) with the integrated system arise from incorporating animals as a new source of protein and economic return into the production system (Oliveira et al., 2014; Martins et al., 2014b; Costa et al., 2014). The increased protein production of integrated systems is only possible with nutrient cycling by animals; in fact, most of the nutrients ingested by grazing are returned to the soil and made available to crops (Sanderson et al., 2013; Alves et al., 2019). Although system fertilization in ICLS increased total forage production, this did not result in higher animal production, thus not resulting in higher production and economic efficiency in these first three years of the experiment. However, it is expected that the higher total production of Italian ryegrass in the long term will benefit the system as a whole and contribute to higher system efficiency.

5. Conclusion

In this short period of time evaluated, system fertilization did not result in greater efficiency in the use of P and K, although it increased the P and K contents of pasture and total herbage production. Integrated system increases nutrient use efficiency, leading to a less negative available K budget in the soil. Neither soybean yield nor sheep live weight gain per unit was influenced by fertilization strategy, but soybean yield was increased by liming. Integrated system increased the use and economic efficiency of P and K fertilization by increasing food production per fertilizer unit. There is evidence that system fertilization provides an effective choice for promoting better use of nutrients by integrated crop-livestock systems, but future studies should continue this approach to evaluate the long-term effect of this fertilizer strategy on the efficiency of use of P and K.

CAPÍTULO III – Biological N_2 fixation by soybeans grown with or without liming on acid soils in a no-till integrated crop–livestock system[3]

1. Introduction

Nitrogen (N) is one of the most critical nutrients required by many field crops (Bender et al., 2015; Fan et al., 2019) such as soybean (*Glycine max* L.), which can accumulate up to 500 kg N ha^{-1} (Balboa et al., 2018; Ciampitti and Salvagiotti, 2018). Soil mineral N and biological N_2 fixation (BNF) are the main N sources for soybeans (Herridge et al., 2008; Peoples et al., 2009). The BNF process delivers the most sustainable N to support plant nutrient demand, reducing the reliance on soil N supply and the mining of the soil N reservoir (Crews and Peoples, 2005). However, under acidic soil conditions, such as low pH and high exchangeable aluminum (Al^{3+}), BNF can be impacted via a decrease in both rhizobium development and plant growth (Sartain and Kamprath, 1975; Alva et al., 1990; Evans et al., 1990).

All around the globe, acid soils represent 50% of the agricultural land (Von Uexküll and Mutert, 1995). In Brazil, it is estimated that 75% of the areas with potential for agricultural activity present problems related to soil acidity (Abreu Junior et al., 2003; Fageria and Baligar, 2003). Additionally, more than 28 million hectares planted with soybeans in Brazil present issues related to soil acidity, hampering the crop attainable yield (CONAB, 2020). Soil acidity is usually characterized by low pH and often severe Al^{3+} toxicity (Sumner and Noble, 2003; Osman, 2018), affecting both root and shoot growth and overall soybean

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productivity (Ferguson et al., 2013; Fageria and Nascente, 2014; Kopittke et al., 2015; Ferguson and Gresshoff, 2015). In a greenhouse study, Lin et al. (2012) found that the number of soybean nodules drastically decreased with soil pH values below 5.5. In addition to soil pH, high concentrations of Al^{3+} also negatively affected soybean nodulation, decreasing both the number and size of nodules (Alva et al., 1990; Lin et al., 2012).

It is well-documented that soybean N demand cannot be met solely via BNF (Ciampitti and Salvagiotti, 2018), and the additional impact of soil acidity forces the crop to become even more reliant on soil N supply resulting in a more negative partial N-balance. Therefore, strategies to mitigate soil acidity are vital. Liming is an agricultural practice widely used in Brazil and it is essential for the country's agricultural production. However, many agricultural areas are still penalized with reduced production due to issues related to soil acidity. Thus, the application of limestone aiming to increase soil pH and exchangeable basic cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), as well as to decrease exchangeable Al^{3+} concentration (Mengel and Kamprath, 1978; Holland et al., 2018; Hale et al., 2020), becomes a critical step to enhance BNF process in soybean grown under acidic soil conditions.

Calcium and Mg are not only essential for plant growth but also critical for the BNF process. Calcium is an essential component to the development of nodules and then to the BNF (Ehrhardt et al., 1996). Magnesium promotes nodule growth in soybeans by facilitating carbohydrate partitioning and transport into nodules (Peng et al., 2018). Thus, in subtropical soils, with low exchangeable Ca^{2+} and Mg^{2+}, liming becomes a key factor to increase soil availability of both nutrients favoring the BNF process.

Decreasing Al^{3+} toxicity is also possible by adding organic residues and increasing soil organic matter content (Sa´ et al., 2009; Miotto et al., 2020). Aluminum can be complexed by low molecular weight organic acids, inhibiting any deleterious effect on cultivated plants (Van Hees et al., 2000; Li and Johnson, 2016; Muhammad et al., 2019). The latter can be attained by implementing integrated crop-livestock systems (ICLS) that increase the addition of both plant and animal residues (Sá et al., 2017; Luz et al., 2019; Alves et al., 2020), resulting in greater soil organic matter relative to traditional grain production systems. This in turn, increases Al complexation by dissolved organic matter, decreasing free Al^{3+} species in soil solution and, thus, its toxicity to plants (Martins et al., 2020).

Recently, with the advancement of the adoption of ICLS in Brazil, new avenues open up with the evolution of ICLS studies, especially regarding the fertilization strategies, such as the concept of “system fertilization”. This new concept is characterized by the anticipation of phosphorus (P) and potassium (K) replacement fertilization to the pasture phase in an ICLS (Farias et al., 2020) instead of applying at soybean sowing (“conventional fertilization”). The addition of P and K at sowing time for soybean crop implies a temporary increase in both P and K soil nutrient availability, lasting only during its growth cycle, with a high level of removal for those nutrients at harvest time. By contrast, application of P and K fertilization for soybean crop well-before during the establishment of the pasture phase can promote a better growth of the pasture, without penalizing soybean yield when the pasture is not grazed or with very low impact when pasture is grazed, with some level of P and K removal from the system via meat production (Alves et al., 2019; Farias et al., 2020). The latter scenario can increase plant residues, and if the pasture is grazed, it promotes even greater root growth and exudation of low molecular weight organic acids (Hamilton et al., 2008; López-Mársico et al., 2015; Sun et al., 2017). Thus, even in soil without liming, less Al^{3+} toxicity to soybean could be expected due to winter grazing in areas receiving “system fertilization” (rather than applied before the summer row crop) potentially due to high Al^{3+} complexation by organic acids, favoring BNF.

Even though several studies have evaluated the effect of soil acidity on legume-root nodulation, there is a lack of studies assessing its effects on BNF, N supply for soybeans and partial N-balance in soils with and without liming. Furthermore, to the extent of our knowledge, there have been no studies examining the additional effect of grazing and fertilization strategies on BNF. Therefore, this study aimed to: (i) quantify the contribution of BNF and soil N pool on the N supply to soybeans, (ii) determine the partial N-balance in the soil, (iii) identify the most relevant soil chemical properties influencing BNF, and (iv) determine soybean seed yield in an acid Acrisol with or without liming, with or without grazing, and managed with conventional or system fertilization.

2. Materials and methods

2.1. Description of the field experiment

A field experiment was carried out at the Experimental Agronomic Station at the Federal University of Rio Grande do Sul, in Eldorado do Sul, Rio Grande do Sul State, southern Brazil (30°05′S, 51°39′W). The regional climate is humid subtropical (Cfa), according to Köppen classification (Alvares et al., 2013), with mean annual air temperature and annual rainfall of 19.4 °C and 1440 mm, respectively. The soil was classified as Acrisol (FAO, 2015). Soil chemical and physical properties at the onset of the study are shown in Table 5.

A preceding experiment was conducted from 2003 to 2017 under no-tillage in an ICLS in the same area. Stocking methods and sheep grazing intensities on Italian ryegrass (*Lolium multiflorum* Lam) in rotation with summer crops (soybean and maize, *Zea mays* L.) were evaluated over a 14-yr period. The area did not receive any soil acidity correction, only fertilization with N, P and K, which dramatically increased acidification over time (Alves et al., 2019).

In 2017 the experimental area of 4.8 ha was rearranged in a factorial model with split plots in a randomized complete block design with four replicates, aiming to test the effect of the animal grazing (non-grazing or ICLS), and fertilization strategies (P and K applied at sowing time for the soybean crop herein termed as “conventional fertilization” or at the sowing of the Italian ryegrass, herein termed as “system fertilization”). In addition, the effect of soil acidity correction (with or without liming) was added in all plots, by excluding areas (4 m × 8 m, totaling 32 m^2) during limestone application to maintain the high acidity conditions (subplot). Soil chemical and physical properties at this time are presented in Table 5. Soil acidity correction using limestone was calculated in order to raise pH to 6.0, as recommended by SBCS (2016). Liming was performed using 7.5 Mg ha^{-1} of dolomitic limestone [$CaMg(CO_3)_2$], with an effective neutralizing power of 72%.

Table 5. Soil chemical and physical properties at the beginning of the field experiment in an Acrisol under no-tillage in Southern Brazil.

Soil layer	pH H_2O [a]	Available [b]		Exchangeable [c]			CEC_{pH7} [d]	Saturation [e]		TOC [f]	Particle-size distribuition [g]		
		P	K	Ca	Mg	Al		Base	Al		Clay	Silt	Sand
cm		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 Al^{3+}, Ca^{2+}, and Mg^{2+}, extracted by 1.0 mol L^{-1} KCl (1:10, v/v).
[d] Cation exchange capacity at pH 7.0, calculated by summing H^+, Al^{3+}, Ca^{2+}, Mg^{2+}, and K^+.
[e] Base saturation = $(Ca^{2+} + Mg^{2+} + K^+)/(CEC_{pH7}) \times 100$, and Al saturation = $Al^{3+}/(CEC_{effective.}) \times 100$.
[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.

The experiment started on July 2017 after Italian ryegrass establishment. Lambs weighing 35 ± 4 kg were continuously stocked (ICLS treatment) to maintain pastures at 15 cm height (considered a moderate grazing intensity). N fertilization was performed using 150 kg N ha^{-1} as urea in all plots. Non-grazed plots had the same Italian ryegrass growing as cover crops. At the end of the pasture phase, sheep were removed, and the grazed and non-grazed areas were desiccated with glyphosate herbicide in preparation for the soybean phase (November – April). The Italian ryegrass was desiccated 15 days before the soybean sowing time.

Two P-K fertilization strategies were evaluated: conventional fertilization, applied at soybean sowing, and system fertilization, applied at the establishment of the Italian ryegrass pasture. The P and K application was calculated by the amount of each nutrient exported by soybean grains based on a targeted yield of 4 Mg ha^{-1}, following recommendations by SBCS (2016). Thus, P and K fertilization was only for replacement of nutrients removed, considering the amount of nutrient exported per ton of soybean seed yield. Fertilizer rates were 30 kg P ha^{-1} of and 58 kg K ha^{-1} (SBCS, 2016). Prior to soybean sowing, seeds were inoculated with *Bradyrhizobium* strains at a dose of 400 ml for every 100 kg of seed. The commercial product used was Gelfix 5® (Nitral Urbana Laboratory, São José dos Pinhais, Paraná State, Brazil) with guarantee of 5 × 109 colony-forming units of *Bradyrhizobium japonicum* per mL. Soybean was sown with 45 cm between rows and at a plant density of 28 plants m^{-2}.

A second pasture-crop sequence was evaluated in 2018 using the same procedures described above. Since soybean phases are present in both years, the evaluated seasons were 2017/18 and 2018/19, each one corresponding to one pasture-crop sequence.

2.2. Soybean nodulation and seed yield

Soybean whole plants were collected at the R1 growth stage (Fehr and Caviness, 1977) in the 2017/18 and 2018/19 cropping seasons for the determination of the shoot and roots dry weight, and number and weight of nodules. The samples were dried at 65 ◦C in a forced-air oven until they reached a constant weight. Six plants were collected randomly on each plot for these determinations.

Soybean seed yield was evaluated over two growing seasons (2017/18 and 2018/19). Yield was evaluated by collecting six random points (2.0 m × 0.45 m) in each plot, totaling 5.4 m^2 (+100 plants per treatment were evaluated for each plot). The samples were dried and grain moisture was determined and adjusted to 130 g kg^{-1}.

2.3. Determination of biological nitrogen fixation (BNF)

At the R7 growth stage of soybean (Fehr and Caviness, 1977) in the 2018/19 cropping season, six plants were randomly sampled on all plots, cut at soil level (stem + leaves + pods). At the same time, six plant of non-N_2-fixing species (*Coniza bonariensis, Solanum americanum, Sida rhombifolia, Urochloa plantaginea*, *Amaranthus hibridus*, and *Bidens pilosa*) were sampled near the plots to be used as reference plants to determine the N derived from the atmosphere (% Ndfa). The biomass samples were dried at 65 °C in a forced air oven until they reached a constant weight to determine the dry weight of the shoot and the N concentration in the shoot. The total N content was obtained by multiplying the shoot dry weight and the N concentration.

We used the ^{15}N natural abundance method (Shearer and Kohl, 1986; Chalk et al., 2016) to estimate BNF in soybean, using non-N_2-fixing plants as a reference. The percentage of BNF (Ndfa) in the N-fixing soybean biomass was calculated though the following equation:

$$Ndfa\ (\%) = [(\delta\ ^{15}N_{nonN} - \delta\ ^{15}N_{Nfix}) / (\delta\ ^{15}N_{nonN} - B\ value)] \times 100 \qquad \textbf{Eq. 4}$$

where δ15NnonN, and δ15NNfix are δ15N values of nodulating and non-N_2-fixing plants, respectively; and the B value used was -2.62‰, the mean of the B values obtained by Guimarães et al. (2008) and Pauferro et al. (2010) for different varieties and inoculants of Brazilian soybean, composed by the *Bradyrhizobium elkanii* and *B. japonicum* strains (Balboa and Ciampitti, 2020). The total N in the shoot derived from N_2 fixation(kg ha^{-1}) was calculated by multiplying the N content on soybean shoots (kg ha^{-1}) by Ndfa (%)/100. Nitrogen derived from the soil was calculated as a difference between the total N and fixed-N in aboveground biomass. Partial N-balance was calculated as the difference between N from BNF and the N exported by soybeans.

2.4. Soil chemical properties

At the same moment of plant sampling (R7 growth stage), soil was collected to determine its chemical properties. The samples were collected with an auger in the 0–20 cm soil layer and dried in a forced-air oven at 45 °C. The larger lumps were broken up, ground and sieved (Ø=2.0 mm). The chemical properties assessed were: pH in water, available P and K extracted by Mehlich-1, exchangeable Al, Ca and Mg extracted by 1.0 mol L^{-1} KCl, base saturation (BS), and Al saturation (AS). Exchangeable Al was determined by titration with 0.0125 mol L^{-1} NaOH solution; Ca and Mg by atomic absorption spectrometry; K by flame photometry. Potential acidity (H+Al) was obtained through the equation proposed by Kaminski et al. (2001), where H+Al is estimated by the pH of equilibrium between soil and the solution with SMP 1.78 mol L^{-1} (triethanolamine, paranitrophenol, K_2CrO_4, $Ca(CH_3COO)_2$ and $CaCl_2.2H_2O$) calibrated at pH 7.5 (Shoemaker et al., 1961). The sum of bases (SB) was determined by the sum of Ca, Mg, and K. The cation exchange capacity at pH 7.0 (CECpH7.0) was calculated by SB + (H + Al); the BS was calculated using the relation: BS = 100 × SB/CECpH7.0; and the AS was obtained by the relation: AS = [Al/(SB + Al)] × 100 (SBCS, 2016).

2.5. Statistical analysis

Statistical analysis was performed with SAS® 9.4 software (SAS Institute, 2015) and the data were subjected to a normality analysis by the Shapiro-Wilk test and the homogeneity of variances by the Levene test, both at a significance level of 5%. A factorial model with split plots were used for the analysis of the number of nodules per plant, nodules dry weight per plant, an average nodules dry weight, shoot dry weight, root dry weight, number of nodules per root dry weight, and soybean seed yield. The effects were of the animal grazing and fertilization strategies, with the liming subdivided in plots, and the year as a repeated measure of time, considering the effects of the block (B), animal grazing (G), fertilization strategies (F), year (Y), the interactions G*F, G*F, G*L, F*L, G*Y, F*Y, L*Y, G*F*L, G*F*Y, G*L*Y, F*L*Y, and G*F*L*Y. The factorial model used subdivided sub-plots for the nitrogen derived from the atmosphere, amount N from N_2 fixation, amount N from soil, plant N content, and partial N balance, considering the effects of the B, G, F, G*F, G*F, G*L, F*L, G*F*L. In two models, the B effect and its interactions were considered random effects and the other factors were considered fixed effects.

The results were submitted to ANOVA at a 5% significance level. When significant, the difference between the treatment means was evaluated by the Tukey test ($p < 0.05$). To evaluate the relationships between variables we used regression analysis (PROC REG) in SAS® 9.4 (SAS Institute, 2015). The relationship between N-fixed and the soil chemical properties (soil pH, exchangeable Ca^{2+}, Mg^{2+} and Al^{3+}, base and Al saturation) was analyzed by multiple regression model in the R software (R Core Team, 2013), testing which variables would have a significant effect on N-fixed. The main soil chemical properties that influence N-fixed were identified using the conditional inference regression tree procedure in the software Jmp® 13 (Jmp, 2015).

3. Results

3.1. Effect of liming on soybean nodulation, development, and seed yield

Of all the factors evaluated in this study, only liming presented a significant effect on the variables analyzed, with neither effect of fertilization strategy nor the sheep grazing (Table 6). For this reason, from now on, the results will be presented especially focused on the liming effect. On average for the two seasons (2017/18 and 2018/19), liming increased the number of nodules (59%; from 75 to 119 per plant), nodules dry weight (64%; from 0.39 to 0.64 g per plant), and number of nodules/root dry weight (28%; 13.3–17 nodules) relative to without liming (Table 6, Fig. 7a, b, and c), all data at plant-level. Shoot and root dry weight also increased by liming, 24% (24.9–30.8 g per plant) and 37% (5.7–7.8 g per plant), respectively ($p < 0.05$; Fig. 8a). Soybean seed yield increased by 11% with liming (2.9 Mg ha^{-1}) versus without liming (2.6 Mg ha^{-1}) (Fig. 8b).

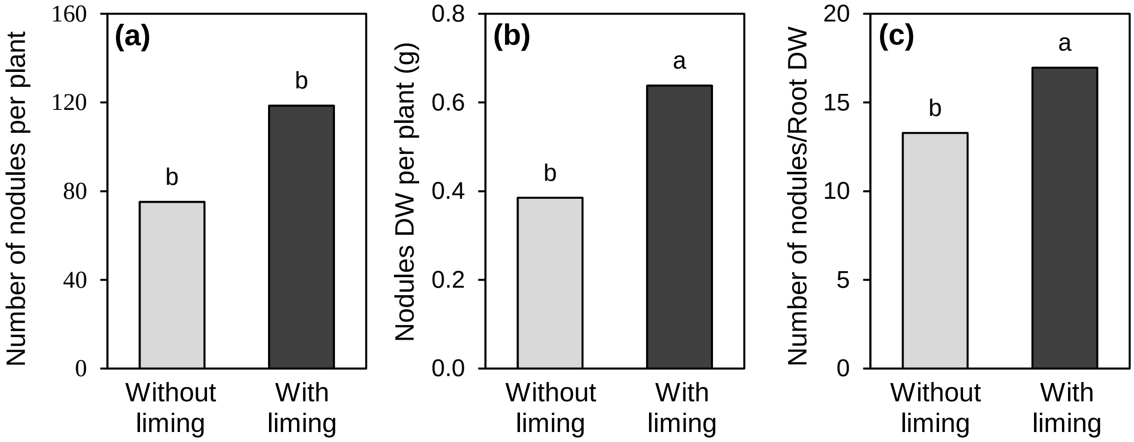

Figure 7. Number of nodules (**a**), nodules dry weight (DW) (**b**), and ratio between number of nodules and root DW (**c**) (average 2017/18 and 2018/19 seasons), all expressed in a plant basis for soybeans grown in an acid Acrisol with or without liming in southern Brazil. The values presented are the overall average of the fertilization strategy and grazing effect (n = 24). Different letters differ by the Tukey test ($p < 0.05$).

3.2. Effect of liming on Ndfa, N-accumulated, and partial N-balance

The overall mean δ ^{15}N value for the reference plants was 5.940‰. For soybean plants, the δ ^{15}N was 25% greater without liming (1.435‰) than in those plants growing in treatments with lime (1.152‰) (Table 7). The average Ndfa in the 2018/19 season was 4% greater without liming (66%) than when liming was applied (62%) (Fig. 9a). However, the total quantity of soybean plant N content from N_2 fixation (fixed-N) was 27% greater with liming (260 kg ha^{-1}) compared to that without liming (205 kg ha^{-1}) (Fig. 9b). Soybean plant N content from soil (soil-N) also increased by 52% (from 104 to 160 kg ha^{-1}) with liming (Fig. 9b). Therefore, N in shoot dry weight was 38% greater with liming (418 kg ha^{-1}) versus without liming (308 kg ha^{-1}) (Fig. 9b).

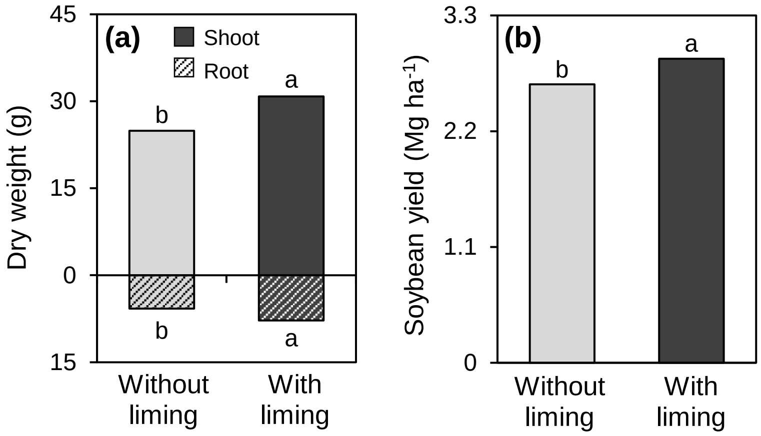

Figure 8. Soybean shoot and root dry weight per plant (**a**), and seed yield (**b**) (average 2017/18 and 2018/19 seasons) of soybeans grown in an acid Acrisol with or without in southern Brazil. The values presented are the overall average of the fertilization strategy and grazing effect (n = 24). Different letters differ by the Tukey test ($p < 0.05$).

Partial N-balance was positive in both treatments, but it was 57% greater under liming application (91 kg ha^{-1}) relative to its counterpart (58 kg ha^{-1}) (Fig. 10a). The relationship between N-balance and Ndfa is presented in Fig. 10b for both treatments, with or without liming. In the limed soil, N-balance increased 12 kg ha^{-1} per unit of Ndfa, versus 4 kg ha^{-1} in the treatment without liming.

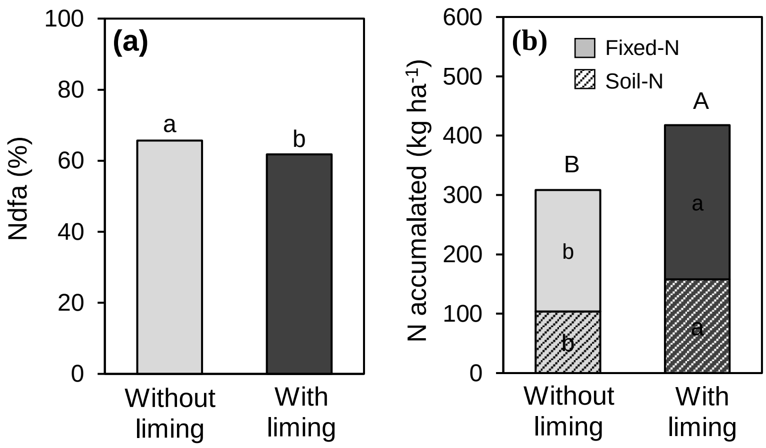

Figure 9. Nitrogen (N) derived from the atmosphere, Ndfa (expressed in percentage, %) (**a**), and N accumulated soil-N and fixed-N (**b**) (only for 2018/19 season) of soybeans grown in an acid Acrisol with or without liming in southern Brazil. The values presented are the overall mean of the fertilization strategy and grazing effect (n = 12). Different letters differ by the Tukey test ($p < 0.05$), where lowercase letters in "b" differentiate the N-fixed and N-soil in treatments with or no liming, and uppercase letters differentiate the N-accumulated (N-fixed + N-soil) in treatments with or no liming.

Table 6. Significance of the effects of experimental factors and their interactions on number of nodules per plant (NN), nodules dry weight per plant (NDW), average nodules dry weight (ADW), shoot dry weight (SDW), root dry weight (RDW), NN/RDW, soybean seed yield (all parameters evaluated 2017/18 and 2018/19 seasons), δ 15N value, nitrogen derived from the atmosphere (Ndfa), amount N from N_2 fixation (N-fixed), amount N from soil (N-soil), plant N content (PNC), and partial N balance (N-balance) (all parameters evaluated only for 2018/19 season) as resulting from analysis of variance (ANOVA).

Experimental factor	NN	NDW	ADW	SDW	RDW	NN/RDW	Yield	$\delta^{15}N$	Ndfa	N-fixed	N-soil	PNC	N-Balance
G [a]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F [b]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L [c]	**	*	ns	*	*	*	*	*	*	**	**	**	**
Y [d]	ns	ns	ns	***	ns	ns	ns	-	-	-	-	-	-
G × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G × L	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F × L	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
F × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
L × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
G × F × L	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G × F × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
G × L × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
F × L × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-
G × F × L × Y	ns	ns	ns	ns	ns	ns	ns	-	-	-	-	-	-

[a] Grazing.
[b] Fertilization season.
[c] Liming.
[d] Year.
[*] Significant at $p < 0.05$.
[**] Significant at $p < 0.01$.
[***] Significant at $p < 0.001$.
[ns] Not significant.

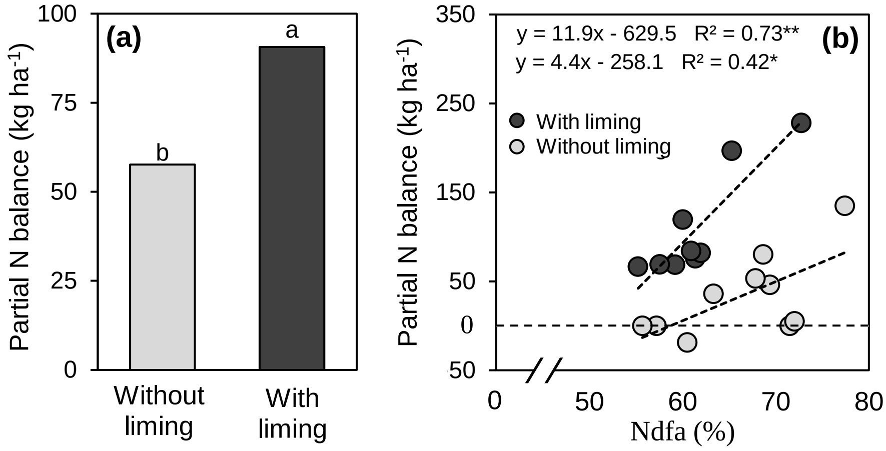

Figure 10. Partial N balance in 2018/19 cropping season (**a**), and relationship between partial N balance and Ndfa (**b**) of soybeans grown in an acid Acrisol with or without liming in Southern Brazil. The values presented are the overall mean of the fertilization strategy and grazing effect (a) (n = 12). The horizontal dashed line in panel b represents neutral (zero) partial N balance. Regression significant at $*p < 0.05$ and $**p < 0.01$.

The Ndfa decreased 0.1% by every unit of N derived from soil (Fig. 11a). Plant N content increased by 1.3 kg ha^{-1} per 1 kg ha^{-1} of N-fixed (Fig. 11b), but the increase was differential in about 12.3 kg ha^{-1} and 8.3 kg ha^{-1} per unit of Ndfa with liming and without liming, respectively (Fig. 11c). Lastly, in overall, soybean yield increased in 5 kg ha^{-1} by every 1 kg ha^{-1} of N-fixed (Fig. 11d).

Table 7. $\delta^{15}N$ values for reference plants and soybean (only for 2018/19 season) in an acid Acrisol with or without liming in southern Brazil.

Reference plants	$\delta\ ^{15}N$ values (‰)
Coniza bonariensis (L.) Cronquist	6.025
Sida rhombifolia L.	6.077
Urochoa plantaginea (Link) Hitch.	5.573
Amaranthus hibridus L.	6.119
Bidens pilosa L.	5.909
Overall mean for reference plants	5.940
Treatments (Soybean - *Glycine max* L.)	
With liming	1.435 a
Without liming	1.152 b

The values presented are the overall mean of the fertilization strategy and grazing effect (n = 12). Different letters differ by the Tukey test ($p < 0.05$).

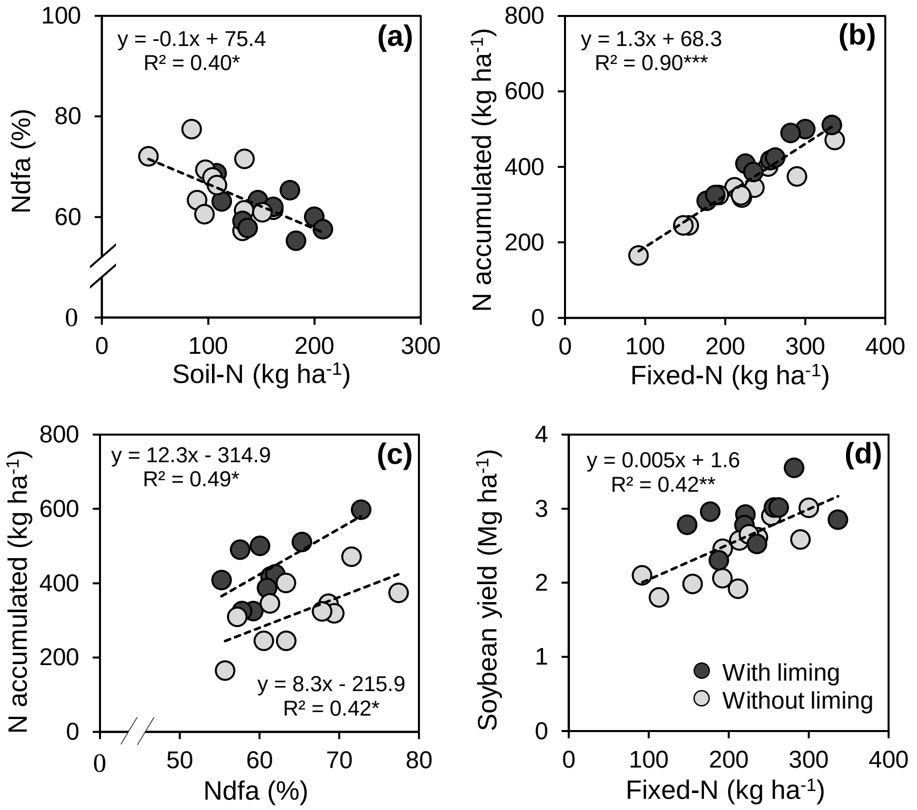

Figure 11. Relationships between nitrogen (N) derived from the atmosphere (Ndfa) and soil-N (**a**), N accumulated and fixed-N (**b**), N accumulated and Ndfa (c), and soybean seed yield and fixed-N (d) of soybeans grown in an acid Acrisol with or without liming in South Brazil (only for 2018/19 season). The values presented are the overall mean of the fertilization strategy and grazing effect. Different letters differ by the Tukey test ($p < 0.05$). Regression significant at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

3.3. Relationship between BNF and soil acidity-related variables

All variables related to soil acidity presented a significant relationship with N fixation evaluated in the 2018/19 season (Fig. 12). Soil pH, base saturation, and exchangeable Ca^{2+} and Mg^{2+} were positively related to fixed-N (Fig. 12a, d, e, and f), while Al saturation and exchangeable Al^{3+} were negatively associated to fixed-N (Fig. 12b and 12c). However, the multiple regression model highlighted that soil pH and Al saturation were the most critical soil chemical properties explaining 76% of the variation of fixed-N (Table 8).

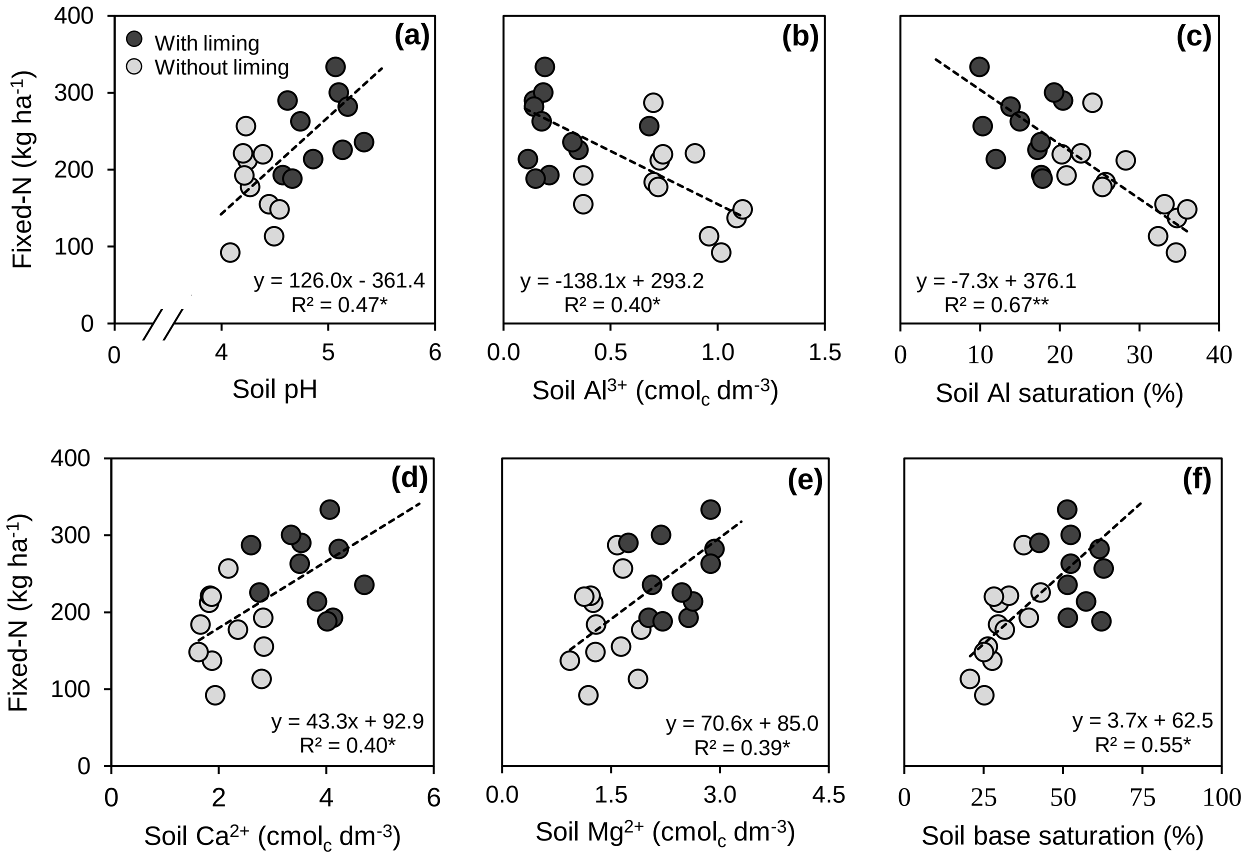

Figure 12. Relationships between fixed-N relative to soil pH (**a**), exchangeable aluminum (Al^{3+}) (**b**), Al saturation (**c**), exchangeable calcium (Ca^{2+}) (**d**), exchangeable magnesium (Mg^{2+}) (**e**), and base saturation (**f**) for soybeans grown in an acid Acrisol with or without liming in southern Brazil (only for 2018/19 season). The evaluated soil layer was 0–20 cm. The values presented are the overall mean of the fertilization strategy and grazing effect. Regression significant at *$p < 0.05$ and **$p < 0.01$.

The regression tree analysis confirmed that pH and Al saturation were the most explanatory soil chemical properties of N-fixed (Fig. 13). For soil pH above 5.1, N-fixed was on average 335 kg ha^{-1}. On the other hand, when the soil pH was lower than 5.1, the amount of N-fixed was dependent on the Al saturation. In this case, when Al saturation was lower than 32%, N-fixed was on average 223 kg ha^{-1}. However, when it was higher than 32%, N-fixed averaged only 129 kg ha^{-1}.

Table 8. Multiple regression model for BNF (% of total N uptake in shoot biomass) as affect by soil chemical properties grown (only for 2018/19 season) in an acid Acrisol with or without liming in southern Brazil.

Explanatory variable	Regression coefficient	Standard error	*p*-value	R^2 (model)
BNF (kg ha^{-1})				0.76
Intercept	-89	148	0.555	
pH	91	28	0.003	
Al saturation	-4.6	1.3	0.002	

4. Discussion

4.1. Effect of liming on soybean nodulation, development, and seed yield

Liming application improved nodule formation and development. Previous studies have already documented that liming increased soil pH (Mengel and Kamprath, 1978; Otieno et al., 2018) and decreased the potential Al toxicity to the host plant (Andrew et al., 1973; Munns et al., 1981; Alva et al., 1987). Low soil pH suppresses nodule development (via receptor kinase GmNARK; Lin et al., 2012), negatively impacting plant and rhizobia growth (Hungria and Stacey, 1997; Morón et al., 2005), and decreasing the synthesis of legume flavonoids and rhizobia Nod factor (McKay and Djordjevic, 1993; Hungria and Stacey, 1997). In addition, the increase of reactive oxygen species and salicylic acid resulting from low pH induced oxidative stress (Borch et al., 1999; Velikova et al., 2000) plays a negative role on nodulation.

Under low soil pH (<5.5), Al^{3+} have an indirect negative effect on nodulation due to its toxicity for soybean plants, thus, reducing both shoot and root growth (Sartain and Kamprath, 1975). Root elongation is impaired by Al phytotoxicity (Kinraide, 1991; Kopittke et al., 2015), with Al^{3+} causing root damage that leads to poor ion and water absorption, also compromising shoot growth (Barceló and Poschenrieder, 2002). Benefits of liming on acidic soils are well known, especially in crop yields (Holland et al., 2019).

4.2. Effect of liming on Ndfa, N-accumulated, and partial N-balance

The relatively lower proportion of Ndfa (%) with liming is due to the better soil conditions provided for soybean root development (Sartain and Kamprath, 1975; Bortoluzzi et al., 2014; Costa et al., 2018), thus, resulting also in a higher rate of root N absorption from the soil. Moreover, the improved growth due to the alleviation of Al^{3+} effects when liming was reflected in a greater quantity of fixed-N and with a positive increment in the overall plant N content. In addition, a proper soil pH increases availability of P, Ca, and Mg (Joris et al., 2013), enhancing plant growth, N demand, N-fixed, and thus, soybean yields.

The benefits of liming can go beyond the correction of soil acidity and increases in crop productivity, directly affecting the partial N-balance in the soil. Partial positive N-balance in the soil is not common. In fact, as reported in the review by Ciampitti and Salvagiotti (2018), positive partial N-balance occurs less than 15% of cases. The positive balance is explained by the lower yield attained in the study, relative to the one reported (5.2 Mg ha^{-1}) for a similar level of fixed-N summarized by Ciampitti and Salvagiotti (2018). The average contribution of Ndfa to soybean in our study was 64%, similar to the mean value of 61% reported by Ambrosini (2019) in a study with 24 sites in the Center-south region of Paraná State, southern Brazil. However, this value is lower than the regional Ndfa range (from 69 to 94%) recorded by other researchers in Brazil (Hungria et al., 2005) and the country-level (80%) as documented by Herridge et al. (2008). Although the Ndfa range between liming treatments (55–73% with, versus 56–77% without) did not vary much, the partial N-balance in the soil was different. In the treatment without liming, a Ndfa below 59% resulted in a negative partial N-balance, but with lime, for this same Ndfa, the partial N-balance in the soil was positive, 69 kg ha^{-1}. The latter scenario reveals the importance of soil correction via liming, not only increasing the supply of essential nutrients but also by benefiting BNF and reducing the negative partial N-balance in the soil.

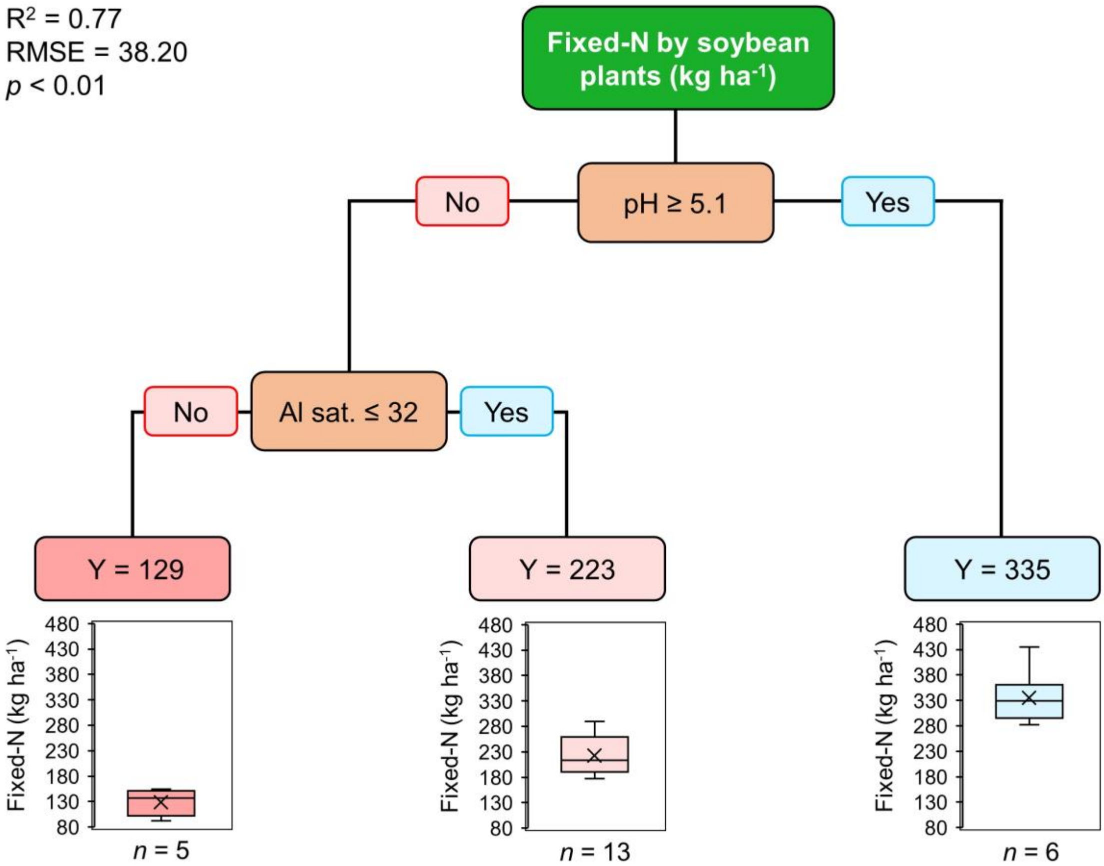

Figure 13. Conditional inference tree for the effect of soil chemical properties (pH and Al saturation) on fixed-N (kg ha^{-1}) in an acid Acrisol with or without liming in Southern Brazil (only for 2018/19 season). The central rectangle of the boxplot spans the first to third input quartile. The solid line inside the rectangle represents the median, the "×" the mean, and vertical lines above and below the box extend to the minimum and maximum values, respectively. The mean effect of soil chemical properties on fixed-N (Y) is shown above the box plot and the number of observations (n) used to calculate the mean effect at the bottom.

4.3. Relationship between BNF and soil acidity-related variables

Our results demonstrated that all chemical properties related to soil acidity affected BNF. Limestone solubilization products consume protons (H^+) from the soil solution, resulting in an increase in soil pH. The increase in soil pH causes precipitation of toxic Al^{3+} and the deprotonation of the functional groups of clay minerals and organic matter in the soil (Rheinheimer et al., 2018a). As a consequence, there is a generation of negative charges on the soil that are immediately occupied by basic cations such as Ca^{2+} and Mg^{2+}, both products of limestone solubilization (Rheinheimer et al., 2018b). Therefore, increasing the contents of exchangeable Ca and Mg and in base saturation (Miotto et al., 2019).

For this reason, interpretation of plants response to liming are wrongly understood by the increase in basic cations (Ca and Mg) concentration or base saturation (Pauletti and Motta, 2019), and even saturation by Ca (Caires and Guimarães, 2018). Although Ca and Mg are essential nutrients, the primary benefit of liming in acid soils is the reduction of Al^{3+} toxicity promoted by the increase in soil pH (Mohr,1950). This primary benefit of lime has been clearly demonstrated in this study, highlighting the most critical chemical properties, soil pH and Al saturation, with the N fixation process. Thus, these parameters should be recommended for the decision-making process of liming. Similar factors are currently considered in the official liming recommendations of the states of Rio Grande do Sul and Santa Catarina, in southern Brazil (SBCS, 2016). Previous studies have also found that soil pH and Al saturation have a direct effect on plant nodulation and growth (Barceló and Poschenrieder, 2002; Lin et al., 2012), directly affecting BNF.

4.4. Effects of grazing and fertilization strategy

The expected benefits of grazing and fertilization system were not confirmed in these first two years of research. From this short-term evaluation, liming is more critical to mitigate the effects of soil acidity and to improve BNF for soybeans than grazing and fertilization strategies. However, Martins et al. (2020) have already demonstrated the benefits of grazing after nine years of ICLS on complexing Al by dissolved organic compounds and decreasing the free Al^{3+} species in soil solution. Improvements induced by ICLS are visible in the long-term, emphasizing the relevance of conducting long-term experiments, even more to document changes in soil quality. Furthermore, future studies should be carried out with different N rates including treatments without N for the Italian ryegrass, in order to evaluate the effect of the N fertilization management of the winter pasture on the BNF, on soil partial N-balance and on soybean yield in the summer.

5. Conclusion

Liming increases soybean seed yield, plant growth, quantity of N-fixed, and root nodulation. Grazing and fertilization strategies did not influence BNF in the first two years of soybean crop. Soil pH and Al saturation are the soil acidity-related properties that most negatively affect the BNF process. Soil liming promotes a more positive partial N-balance in soybean, contributing N to the rotation, reducing the dependency on synthetic N fertilizers and the mining of soil N reservoir from soil organic matter, and increasing the long-term sustainability and resiliency of this ICLS.

CAPÍTULO IV – Grazing, liming, and fertilization: shifts on soil fertility and microbial community in a no-till sheep-soybean integrated system[4]

1. Introduction

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

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[4] Published in the *Applied Soil Ecology* (Alves et al. 2023, doi: 10.1016/j.apsoil.2023.104893)

In ICLS, the animal grazing component induces greater root growth of pastures and can positively affect the soil microbial community (López-Mársico et al., 2015). Besides grazing, other practices, such as liming, can impact the soil microbial community. Soil pH is critical for the soil microbial community (Wu et al., 2017; Wan et al., 2020). Bacterial and fungal populations are affected by soil pH. Gram-positive (Gram+) bacteria and fungi appear to be more tolerant to low soil pH, high aluminum (Al) concentrations, and low C inputs than Gram-negative (Gram–) bacteria (Pietri & Brookes, 2009). In addition to the direct effects of improving soil chemical properties in acid soils (Holland et al., 2018; Hale et al., 2020), liming contributes to higher root and shoot biomass production due to a better environment for plant growth (Alves et al., 2021), as well as providing a better environment for the soil microbial community (Treonis et al., 2004; Sen et al., 2022). However, the impact of liming on the soil microbial community in acidic, highly weathered agricultural soils is limited.

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

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

2. Material and methods

2.1. Site description and experimental design

The study site was located at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul, in Eldorado do Sul, Rio Grande do Sul State, Brazil (30°05′ S, 51°39′ W, 46 m above sea level). The regional climate is humid subtropical (Cfa), according to the Köppen classification (Alvares et al., 2013), the regional climate is humid subtropical (Cfa), with mean annual air temperature and precipitation of 19.4 °C and 1440 mm, respectively. The soil was classified as Acrisol (FAO, 2015). Soil chemical and physical properties of the study site are described in **Table 9**.

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

The split-plot was the effect of lime application (i.e., surface liming and a control with no lime). The effect of soil acidity neutralization (i.e., with or without lime) was examined in all plots by excluding an area of 32 m^2 (4 m × 8 m) from lime application to maintain the original acidity conditions. Soil acidity was amended by applying lime in the amount needed to raise pH to 6.0 according to regional recommendations (SBCS, 2016). The soil was limed with 7.5 Mg ha^{-1} of dolomitic lime [$CaMg(CO_3)_2$] with an effective neutralizing power of 72%. Liming was applied in July 2017 without incorporation.

Table 9. Soil chemical and physical properties at the beginning of the field experiment in an Acrisol under no-tillage in Southern Brazil.

Soil layer	pH H_2O [a]	Available [b]		Exchangeable [c]			CEC_{pH7} [d]	Saturation [e]		TOC [f]	Particle-size distribuition [g]		
		P	K	Ca	Mg	Al		Cation	Al		Clay	Silt	Sand
cm		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 Al^{3+}, Ca^{2+}, and Mg^{2+}, extracted by 1.0 mol L^{-1} KCl (1:10, v/v).
[d] Cation exchange capacity at pH 7.0, calculated by summing H^+, Al^{3+}, Ca^{2+}, Mg^{2+}, and K^+.
[e] Cation saturation = $(Ca^{2+} + Mg^{2+} + K^+)/(CEC_{pH7}) \times 100$, and Al saturation = $Al^{3+}/(CEC_{effective.}) \times 100$.
[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.

The sheep grazing started in June and finished in October in 2017 and 2018 (Table 2). Italian ryegrass was established by broadcasting viable seeds at a rate of 25 kg ha^{-1} with a centrifugal distributor in May of each year. Urea (45% N) was applied at 150 kg N ha^{-1} to all paddocks at the Italian ryegrass V3 stage (3 totally expanded leaves). **Table 10** contains detailed information about the animals. A variable number of put-and-take animals (Mott and Lucas, 1952) was used to maintain the desired sward canopy height (SCH). This method was used to adjust the stocking rate and maintain the average SCH at 15 cm, which provides the optimum plant structure for maximizing animal production (Carvalho, 2013). The SCH was monitored at 7-day intervals by using a sward stick (Barthram, 1985) for the measurement of 150 randomly chosen points per experimental unit monthly. At the end of the pasture phase, residual ryegrass was terminated with glyphosate herbicide before soybean planting.

Table 10. Details of the pasture and cropping phases in the first three years of the experiment.

Pasture phase	Season	
	2017	2018
Stocking period		
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
Animal information		
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.

The P and K fertilizer rates were calculated according to SBCS (2016), which considers that when soil P and K levels are above high, only replace nutrients removed. The rates were calculated from the amounts of P and K removed by soybean with an expected yield of 4.0 Mg ha^{-1} (Table 2). Before soybean planting, seeds were inoculated with *Bradyrhizobium* strains at a dose of 400 ml for every 100 kg of seed. The commercial product was Gelfix 5® with a guarantee of 5×10^9 colony-forming units of *Bradyrhizobium japonicum* per mL. Properly inoculated soybean seeds do not require N fertilization (SBCS, 2016). Soybean row spacing was 45 cm. **C**ultivars, planting method, harvesting date, and planting density for the 2017/2018 and 2018/2019 seasons are presented in **Table 10**.

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

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

Another part of the soil samples was dried in a forced-air oven at 45 °C, the larger lumps crumbled, ground, and sieved (Ø = 2.0 mm) for determination of soil chemical properties. Soil pH was determined in water suspension at a 1:1 soil-water ratio in a benchtop pH meter model Digimed DM-22®. Soil available P and K were extracted with Mehlich-1 solution. Mehlich-P was analyzed colorimetry (Femto 600 Plus® colorimeter). Mehlich-K was analyzed by emission (Digimed NK-2000® flame photometer). Exchangeable calcium (Ca), magnesium (Mg), and Al were extracted with KCl 1.0 mol L^{-1}, Ca and Mg analyzed AA spectrometry (PerkinElmer AAnalyst 200® atomic absorption spectrometer). Extractable Al was analyzed by titration with NaOH 0.0125 mol L^{-1} solution (Tedesco et al., 1995). The sum of exchangeable cations (SC) was determined by summing Ca, Mg, and K. The cation exchange capacity at pH 7.0 ($CEC_{pH7.0}$) was calculated by SC + (H+Al). Cation saturation was calculated as V (%) = $(SC)/CECpH_{7.0} \times 100$; and Al saturation as m (%) = [Al/(Ca+Mg+K+Al)] × 100 (SBCS, 2016).

The soil microbial community composition was assessed by analyzing the phospholipid fatty acids (PLFA) composition, which was performed using freeze-dried samples. Total lipids were extracted using a modification of the Bligh and Dyer (1959) extraction (White and Rice, 2009). Total lipids were extracted by 10 mL of methanol (25.0 mol L^{-1}), 5 mL of chloroform (12.0 mol L^{-1}), and 4 mL of phosphate buffer (0.5 mol L^{-1}) at pH 7.4 on 5 g freeze-dried soil. Nanopure water (5 mL) and chloroform (5 mL) were added after 3 hours to separate the mixture into polar and non-polar fractions, whereas total lipids remained in the non-polar phase. Phospholipids were isolated from neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®, Avantor, Radnor, Pennsylvania, USA) and eluted with methanol. The phospholipids were then saponified by KOH, methylated to fatty acid methyl esters (FAME), and analyzed by Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30 m x 250 μm in diameter x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). Helium was used as carrier gas. FAME peaks were recognized by different retention times in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1,114; Matreya LLC, Pleasant Gap, Pennsylvania, USA).

Sample peaks were quantified based on a comparison of the abundance with an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g^{-1} dry soil. **Table 11** shows the groupings of fatty acids and their respective microbial groups. The total microbial biomass estimate from PLFA was determined as the sum of Gram+, Gram–, actinomycetes, saprophytic fungi, and arbuscular mycorrhizal fungi (AMF), except nematodes and protozoa, which were not determined by the method.

Table 11. 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; 16:1ω7
Actinomycetes	10-methyl 18:0; 10-methyl 19:0
Saprophytic fungi	18:2ω9, 12
AMF[(1)]	16:1ω5

[(1)]Arbuscular mycorrhizal fungi.

Permanganate oxidable carbon (POxC), also known as active carbon, was measured following the procedure of Weil et al. (2003). Briefly, 2.5 g soil was mixed with 18 mL of deionized water and 2 mL of 0.2 M $KMnO_4$, then shaken for 2 min and allowed to settle for 10 min. Following settling, 0.5 mL of the supernatant was transferred into another tube and mixed with 49.5 mL of deionized water. Absorbance was measured in a spectrophotometer at 555 nm. The change in $KMnO_4$ concentration was used to estimate the amount of oxidized C, assuming that 1 mM $KMnO_4$ is consumed in the oxidation of 0.75 mM or 9 mg of C.

2.3. Statistical analysis

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

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

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

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

3. Results

3.1. Soil chemical properties

Soil chemical properties changed as a function of soil liming (Table 12). Soil liming increased soil pH from 4.5 to 5.5, exchangeable Ca content from 2.2 to 3.9 $cmol_c$ dm^{-3}, exchangeable Mg content from 1.4 to 2.6 $cmol_c$ dm^{-3}, and cation saturation from 33 to 53% at 0-5 cm soil layer, compared to no lime application (Table 13). At the same layer, lime application decreased the exchangeable Al from 0.8 to 0.2 $cmol_c$ dm^{-3} and Al saturation from 17 to 4%, compared to no lime (Table 13). On the other hand, at 10–15 only exchangeable Ca content was changed by liming. The limed treatment had an exchangeable Ca content of 1.5 $cmol_c$ dm^{-3} compared to 1.0 $cmol_c$ dm^{-3} in the unlimed treatment (Table 13). Fertilization strategy and sheep grazing did not change the evaluated soil chemical properties.

3.2. Soil microbial community and labile carbon

The soil microbial community changed as a function of soil liming, sheep grazing, and the interaction among lime, fertilization strategy, and sheep grazing. However, the liming effect was restricted only to the 0–5 cm soil layer and did not influence the subsurface 10–15 cm soil layer (Table 14). Soil liming decreased total microbial biomass (–27%, from 45.5 to 33.2 nmol PLFA g^{-1} soil, Fig. 14a), the total bacterial community (–29%, from 20.7 to 14. 6 nmol PLFA g^{-1} soil, Fig. 14b), Gram+ bacteria (–29%, from 14.7 to 10.4 nmol PLFA g^{-1} soil, Fig. 1c), and actinomycetes (–21%, from 2.4 to 1.9 nmol PLFA g^{-1} soil, Fig. 14d), but increased the fungi:bacteria ratio (+17%, 0.15 to 0.18, Fig. 14e) at 0–5 cm soil layer.

Table 12. 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
	------------------------------------- *Soil layer of 0–5 cm* -------------------------------------							
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
	------------------------------------ *Soil layer of 10–15 cm* ------------------------------------							
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.

Sheep grazing increased the saprophytic fungi community by 50% in the 10–15 cm soil layer (Fig. 14f). The interaction among sheep grazing, fertilization strategy, and liming changed the AMF community at the 10–15 cm soil layer (Table 15). In the integrated system with system fertilization, liming increased AMF 2.4 times compared to the treatment without liming (1.2 and 0.5 nmol PLFA g^{-1} soil, respectively). Furthermore, at the 10–15 cm soil layer, the integrated system with liming and system fertilization increased AMF by 3.0-fold compared to conventional fertilization (1.2 and 0.4 nmol PLFA g^{-1} soil, respectively). The combination of liming and system fertilization in the integrated system increased the AMF community 3.0-fold compared to the specialized system (1.2 and 0.4 nmol PLFA g^{-1} soil, respectively). Other comparisons were not significant (Table 15).

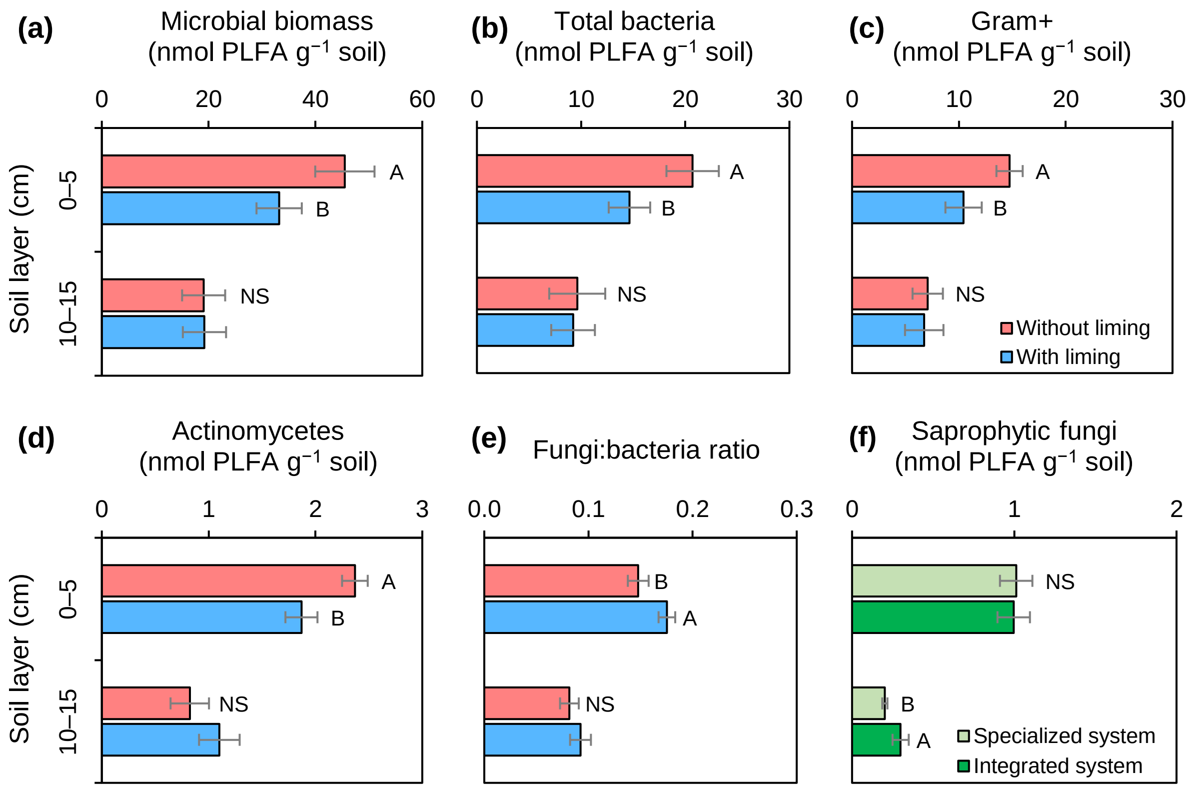

Figure 14. Microbial biomass (**a**), total bacteria (**b**), bacteria gram+ (**c**), actinomycetes (**d**), and fungi:bacteria ratio (**e**) as a function of liming, and saprophytic fungi (**f**) as a function of animal grazing in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Different letters differentiate the treatments within each layer by Tukey's test at a 5% significance level. NS = not significant.

Table 13. 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
Ca^{2+} ($cmol_c$ dm^{-3})	2.2 ± 0.6 B	3.9 ± 0.7 A	1.0 ± 0.1 B	1.5 ±0.3 A
Mg^{2+} ($cmol_c$ dm^{-3})	1.4 ± 0.3 B	2.6 ± 0.5 A	1.1 ± 0.4	1.1 ± 0.4 ns
Al^{3+} ($cmol_c$ 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

3.3. Principal component analysis, Pearson’s correlation, and the relationship between soil chemical and biological properties

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

These results were supported by the significant correlations between soil chemical properties and the microbial community (Fig. 16). Pearson's correlation matrix highlighted the positive and negative correlation between soil chemical properties and the soil microbial community, which were distinct for the 0–5 and 10–15 cm soil layers. For the surface (0–5 cm), soil microbial biomass decreased as a function of increasing pH (r = –0.68), cation saturation (r = –0.48) and exchangeable Ca (r = –0.44) and Mg (r = –0.59) concentrations and increased as a function of Al saturation (r = 0.43) and K availability (r = 0.68) (Fig. 16a). In contrast, in the subsurface the microbial biomass increased as a function of pH (r = 0.41), cation saturation (r = 0.43), and exchangeable Ca (r = 0.41) and Mg (r = 0.49) concentration (Fig. 3B). Furthermore, at the 0–5 cm soil layer there was a negative correlation between soil pH, cation saturation, and exchangeable Ca and Mg concentration with Gram+ bacteria population (Fig. 16a). All these correlations were significant ($p < 0.05$).

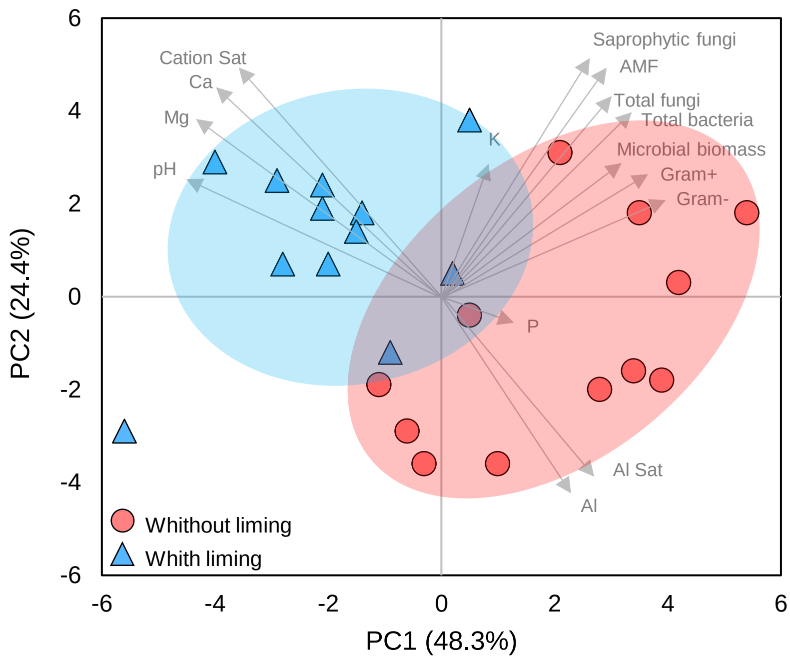

Figure 15. Scatter plot of principal component analysis (PCA) of microbial community by soil chemical properties at 0–5 cm soil layer in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil.

Similarly, at the 0–5 cm layer, we observed a negative relationship between soil pH (Fig. 17a), cation saturation (Fig. 17b), exchangeable Ca (Fig. 1d) and Mg (Fig. 17e) concentration, and positive for Al saturation (Fig. 17c) and available K contents (Fig. 17f) with microbial biomass. There was a differentiation for the 10–15 cm layer, where soil pH (Fig. 17a), cation saturation (Fig. 17b), exchangeable Ca (Fig. 17d) and Mg (Fig. 17e) had a positive relationship with soil microbial biomass. Furthermore, a negative relationship was observed between soil pH (Fig. 18a), cation saturation (Fig. 18b) and exchangeable Ca (Fig. 18c) and Mg (Fig. 18d) concentration and Gram+ bacteria.

Table 14. 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.

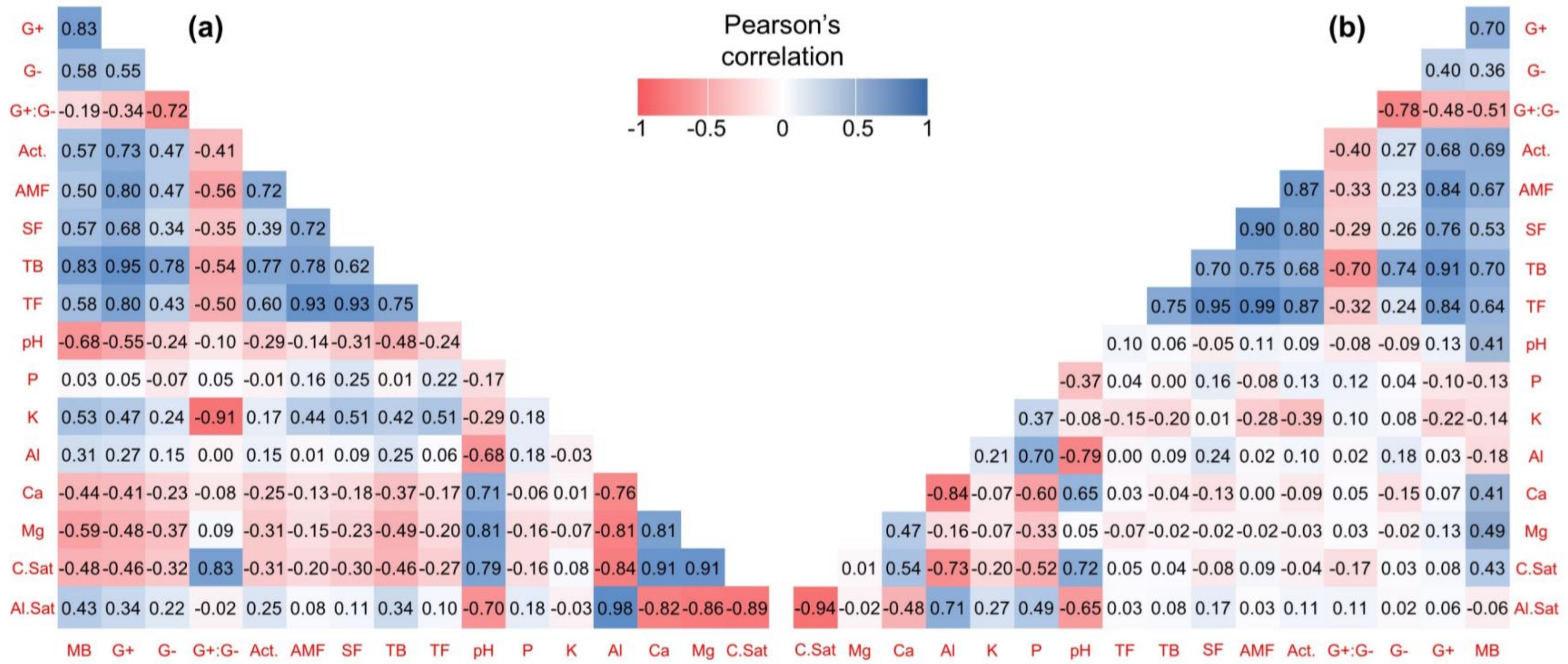

Figure 16. Person's correlation coefficient between soil chemical properties (pH = soil pH, P = phosphorus, K = potassium, Al = exchangeable aluminum, Ca = exchangeable calcium, Mg = exchangeable magnesium, C. Sat = cation saturation, and Al Sat. = aluminum saturation) and microbial communities (MB = microbial biomass, G+ = gram+ bacteria, G- = gram- bacteria, G+:G- = gram+:gram- ratio, Act. = actinomycetes, AMF = arbuscular mycorrhizal fungi, SF = saprophytic fungi, TB = total bacteria, and TF = total fungi), in 0–5 cm (**a**) and 10–15 cm (**b**) soil layers in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil.

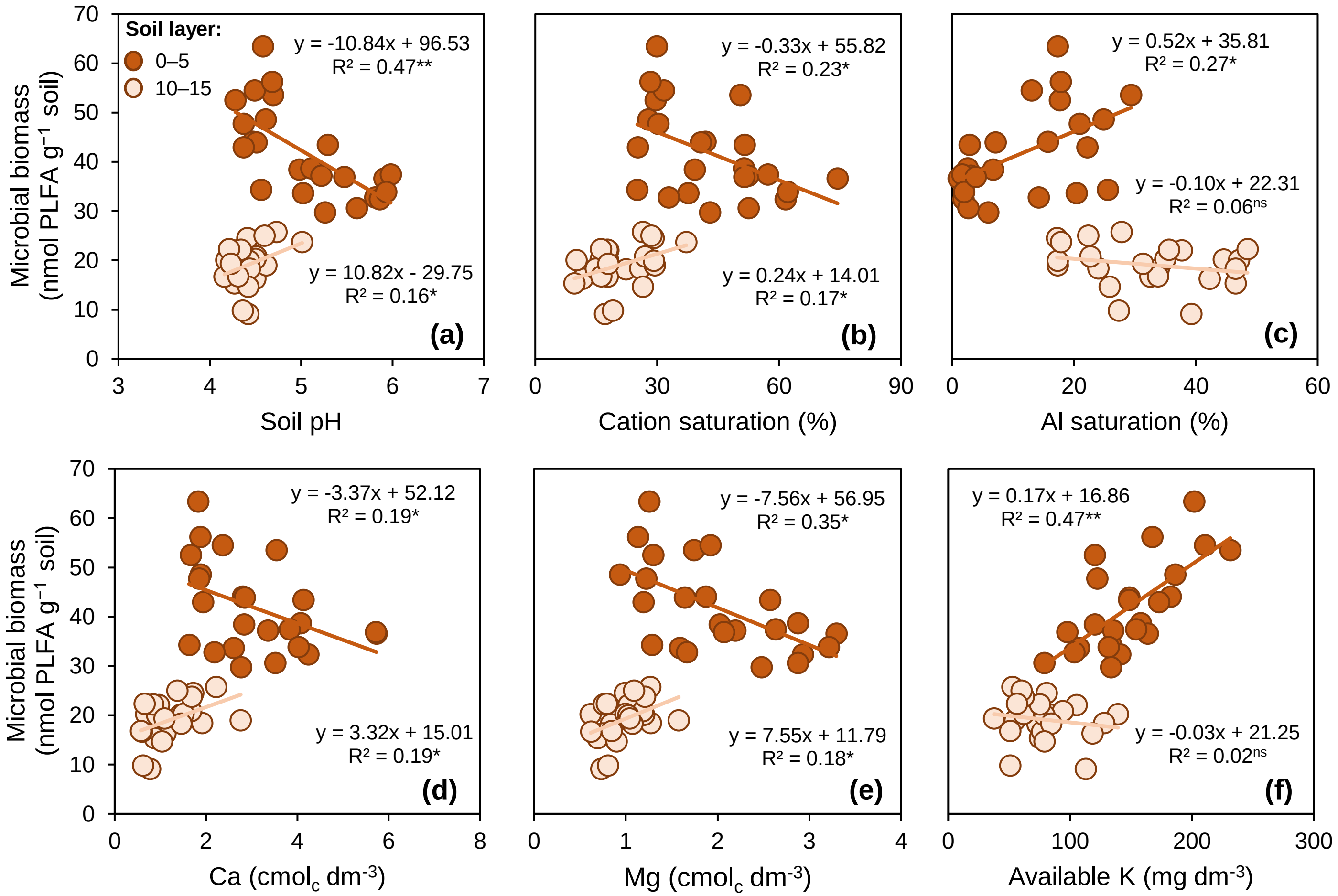

Figure 17. Relationships between microbial biomass relative to soil pH (**a**), cation saturation (**b**), Al saturation (**c**), exchangeable calcium (Ca) (**d**), exchangeable magnesium (Mg) (**e**), and available potassium (K) (**f**) in soil layers of 0–5 and 10–15 cm in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Regression were significant at $^{*}p < 0.05$ and $^{**}p < 0.01$.

Table 15. 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
	----------------------------	*nmol PLFA g^{-1} soil* ----------------------------		
Without	0.5 ± 0.4 Aa*a*	0.5 ± 0.1 Aa*a*	0.7 ± 0.2 Aa*a*	0.5 ± 0.1 Ba*a*
With	0.7 ± 0.3 Aa*a*	0.4 ± 0.2 Aa*b*	0.4 ± 0.3 Ab*a*	1.2 ± 0.5 Aa*a*

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.

4. Discussion

Liming improved soil chemical properties by increasing pH, exchangeable Ca and Mg content, and decreasing exchangeable Al, consequently affecting the soil microbial community. Contrary to previous results studies (Kennedy et al., 2004; Xue et al., 2010; Li et al., 2022), we found a negative effect of pH increase (Fig. 17a) on soil microbial community at the surface soil layer, where lime application resulted in lower microbial biomass (Fig. 14a), and total bacterial biomass (Fig. 14b). Although a modest stimulus in the soil microbial community was noticed after lime application, the microbial biomass did not change or even decreased due to the reduced of SOC after 15 days (Badalucco et al, 1992; Wachendorf, 2015). The decrease in total bacteria was mainly a result of the decrease in Gram+ bacteria (Fig. 14c), which represents the largest portion of the total bacteria and a large part of the total microbial biomass, along with actinomycetes (Fig. 14d). Our finding corroborates with previous studies, demonstrating a negative relationship between soil pH and abundance of cy19:0, i16:0, i17:0, fatty acids respective to Gram+ bacteria (Frostegård et al., 1993; Bååth and Anderson, 2003; Rousk et al., 2010a, b; Abalos et al., 2020).

On the other hand, a positive relationship was observed between soil acidity-related properties such as pH, cation saturation, Al saturation, and exchangeable Ca and Mg with microbial biomass in the subsurface soil layer from 10–15 cm, differing from the effect observed in the superficial 0–5 cm soil layer, which was decreased (Fig. 17a, b, c, d, and e). These differing results observed between the surface (0–5 cm) and the subsurface (10–15 cm) can be explained by the contribution of limestone to SOC mineralization rate. Liming and subsequent elevation in soil pH, may indirectly increase microbial activity initially by stimulating SOC decomposition (Andersson et al., 2000; Pietri and Brookes, 2008; Garbuio et al., 2011). A higher SOC mineralization rate was observed in a soil incubation experiment (Xiao et al., 2018), possibly linked to the increased microbial activity due to $CaCO_3$ application, depleting the available substrate and contributing to a gradual decrease in soil microbial biomass at the same level as the control (Feng et al., 2016). However, in our results, this effect was observed only at the surface because the alkalinizing effect of the lime was restricted to the first few centimeters, not altering the subsurface (Table 13). Thus, we have a different behavior between the surface and subsurface regarding soil microbial community and soil chemical properties, as observed in Fig. 16.

Liming did not alter the two groups of fungi represented by the PLFA biomarkers in the surface (0–5 cm of soil layer), suggesting that the fungal community was less sensitive to soil pH changes. Our result were supported by Rousk et al. (2010a,b), who observed a positive effect of increasing soil pH (from 4 to 8) in the soil microbial community but no effect on the fungal community. A positive effect between lime application, system fertilization, and grazing was observed on the AMF community in the subsurface (10–15 cm of soil layer) (Table 15). This result can be likely due to AMF being strongly influenced by plant-derived C (Nurlaeny et al. 1996; Tavi et al., 2013; Cui et al., 2018), which was higher under lime versus without lime treatments (Nurlaeny et al. 1996; Alves et al., 2021). In addition to liming, improved pasture nutrition with P and K fertilizers to the pasture phase (system fertilization) promoted higher biomass production of Italian ryegrass (Farias et al., 2020; Alves et al., 2022). Thus, the greater C inputs through the contribution of pasture under system fertilization compared to conventional fertilization likely favored the AMF community. In addition, in the integrated system, the input of the animal manure and the induction of greater root growth by grazing (López-Mársico et al., 2015) may provide a more suitable environment with higher organic C source for fungal development for, both AMF and saprophytic fungi (Fig. 14f). This increase in the fungal biomass in the subsurface play an important role in soil C stabilization as they physically entangle micro-aggregates to form macro-aggregates, and release cohesive substances such as glycoproteins (glomalin) that boosts aggregation (Wilson et al., 2009; Veloso et al., 2020).

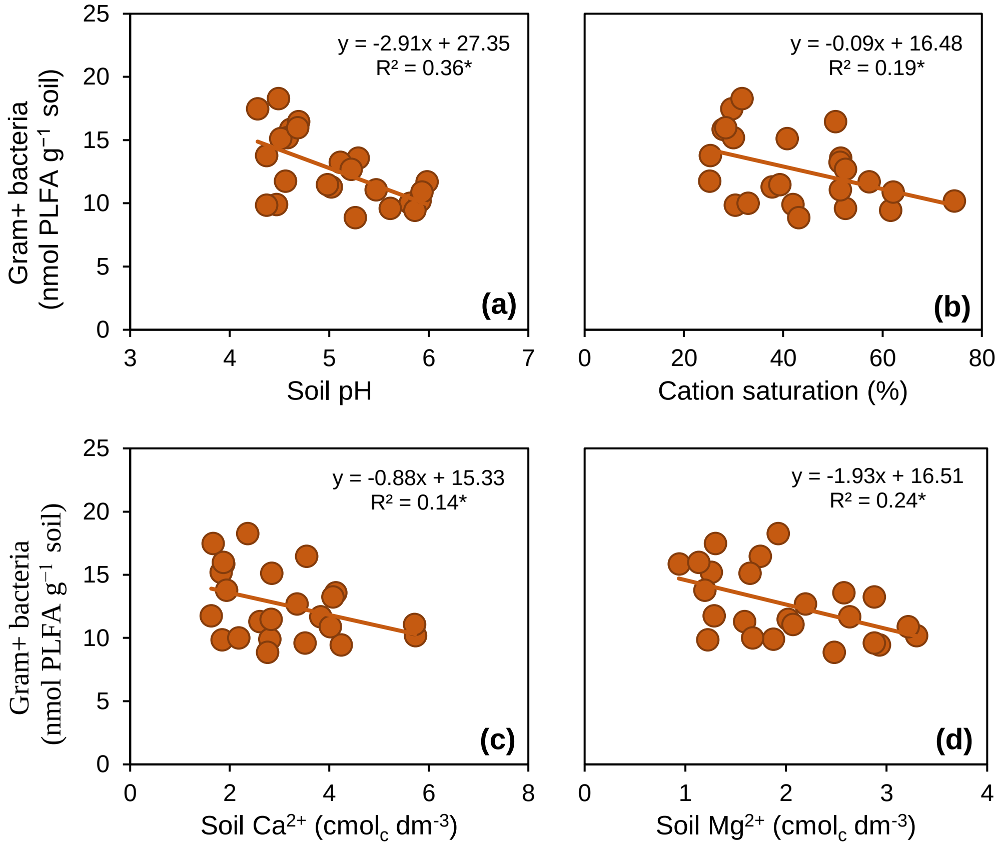

Figure 18. Relationships between gram+ bacteria to soil pH (**a**), cation saturation (**b**), exchangeable calcium (Ca^{2+}) (**c**), and exchangeable magnesium (Mg^{2+}) (**d**) in soil layer of 0–5 cm in an experiment under integrated crop-livestock system with soybean and sheep in Southern Brazil. Regression were significant at $*p< 0.05$.

Fine roots and AMF often represent the largest C input into soils (Kramer et al. 2010; Clemmensen et al. 2013), and their fate is therefore of primary importance for SOC balance, highlighting the importance of increasing the population of these organisms in deeper soil layers for subsurface C increment. The AMFs are the dominant mycorrhizal type in grasslands (Read and Perez-Moreno 2003), and their increase can be of interest, especially in ICLS, since in these systems the soil was covered by grasses. Furthermore, by moving nutrients from the soil to plant roots in exchange for photosynthate, AMF are the agents directing C to the soil (Johnson et al. 2013). Besides better utilization of mineral nutrients, the deposition of fungal material can increase soil aggregation that can prevent the decomposition of soil organic matter (Wilson et al. 2009).

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

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

5. Conclusion

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

CAPÍTULO V – Conclusões e considerações finais

Os estudos desenvolvidos na presente tese trouxeram resultados inéditos relacionados a temática da adubação de sistemas, demostrando seu potencial de utilização, principalmente quando adotados em sistemas que envolvam a entrada do componente animal em pastejo, caracterizando um sistema integrado de produção. Levando em consideração os aspectos produtivos, a adoção da estratégia de fertilização de sistema proporciona maior produção de forragem, não se diferencia da fertilização convencional para a produtividade da soja e a produção animal. Cabe ressaltar, que antes mesmo de pensarmos no melhor sistema de produção e estratégia de fertilização, a correção da acidez é indispensável para o sucesso produtivo de sistemas agrícolas conduzidos sob solos ácidos.

Apesar de ser um tema amplamente discutido na literatura, a calagem em sistemas agrícolas ainda é um temática de destaque na atualidade. Nesse sentido, pode-se observar resultados inéditos relacionados aos efeitos da acidez e sua correção pela calagem na FBN na soja, demostrando que o momento que elevamos o pH do solo e diminuímos a saturação por Al, o montante de nitrogênio fixado pelas bactérias pode ser aumentado em 160%, além de aumentar a produtividade da cultura.

Além dos efeitos em isolado, podemos observar o sinergismo dos tratamentos, principalmente quando se refere a comunidade de fungos no solo. A combinação de um sistema integrado, fertilização de sistema e calagem aumenta a comunidade de fungos micorrízicos arbusculares em três vezes em relação a um sistema especializado com fertilização convencional na camada subsuperficial do solo, demostrando que a interação dos fatores promoveram melhores condições de desenvolvimento a esse tipo de comunidade. Além disso, o pastejo ovino aumenta a biomassa de fungos saprofíticos em 50% na camada subsuperficial do solo, em comparação com o sistema especializado.

Assim, acreditamos que nossos estudos venham a colaborar para os avanços em relação ao manejo da calagem, estratégias de fertilização em sistemas integrados sobre os aspectos produtivos e do manejo sustentável do solo. Servindo assim como base para tomada de decisão sobre o melhor manejo da fertilização e calagem em sistemas integrados de produção.

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Apêndices

1. Artigo publicado na *Revista Brasileira de Ciência do Solo* referente ao segundo capítulo da presente tese.

Rev Bras Cienc Solo 2022;46:e0210125 Article

Revista Brasileira de Ciência do Solo

Division - Soil Use and Management | Commission - Soil Fertility and Plant Nutrition

Fertilization strategies and liming in no-till integrated crop-livestock systems: effects on phosphorus and potassium use efficiency

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ABSTRACT: In an integrated crop-livestock system (ICLS), system fertilization exploits the nutrient cycling imposed by animal grazing and increases the system efficiency. An increasingly popular approach to fertilization in southern Brazil is anticipating P and K requirements for soybeans into the pasture phase. This can increase the use efficiency of these nutrients in ICLS based on meat production in winter and soybean in summer. This study aimed to evaluate the effect of fertilization strategy, grazing and soil acidity correction on herbage and animal production, soybean yield, P and K contents in soil and plant tissue, and P and K use and economic efficiency. In 2017, a field experiment was established on an Acrisol (*Argissolo Vermelho distrófico*) double-cropped with soybean and Italian ryegrass under no-tillage. Herbage and animal production, soybean yield, available P and K contents, and P and K plant tissue status were determined. Available P and K in the soil were unaffected by grazing and fertilization strategy. Conversely, system fertilization and liming increased the P and K contents of aboveground Italian ryegrass biomass. Additionally, the available K budget in the soil was 2.7 times smaller in the integrated system with system fertilization than in the specialized system with conventional fertilization, possibly due to K fixation in non-exchangeable forms. By contrast, the available P budget in the soil was not affected by treatments and was positive with all systems. The use of ICLS increased economic return, and P and K use efficiency for protein production. System fertilization did not affect soybean yield, but it increased the total herbage production of Italian ryegrass. Despite this, sheep live weight did not increase. Using ICLS in combination with system fertilization provides an effective nutrient management strategy with a higher potential for sustainable food production when compared with conventional fertilization.

Keywords: soybean yield, sheep grazing, animal production, nutrient management.

2. Artigo publicado na *Soil & Tillage Research* referente ao terceiro capítulo da presente tese.

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Biological N_2 fixation by soybeans grown with or without liming on acid soils in a no-till integrated crop-livestock system

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A R T I C L E I N F O

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Low pH
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A B S T R A C T

Soil acidity can impair biological N_2 fixation (BNF) and increase soybean (*Glycine max* L.) reliance on soil N to satisfy its plant N demand. This study aimed to evaluate the effect of liming, fertilization strategy, and grazing on: i) the N supplied *via* BNF process, ii) partial N-balance, and iii) soybean yield, in an integrated crop-livestock system under a non-tilled sandy and acidic Acrisol. Initial soil conditions were soil pH of 4.0 and Al saturation of 50%. Limestone was applied to rise soil pH to 6.0 and a control area was kept with the initial soil conditions. Soybean was grown in the summer and Italian ryegrass (as a cover crop or grazed by sheep) in the winter. Fertilization strategies consisted in applying P and K prior to soybean or ryegrass. Partial N-balance was calculated by the difference between N derived from BNF and N removed in soybean seeds. Contribution of N from the atmosphere to soybeans was on average 62 and 66% in plots without and with liming, respectively. The fixed-N was 27% greater under low soil acidity due to lime. Partial N-balance was positive in both treatments, but 57% greater in the limed- relative to the non-limed (+58 kg ha^{-1}) soils, resulting in 11% soybean yield increase when lime was added. The most relevant soil chemical properties influencing N fixation were soil pH and Al saturation. Grazing and fertilization strategies had no effect on soil acidity properties, N supply, and soybean evaluations. Further research should explore the long-term effect of grazing, fertilization strategies, and liming.

1. Introduction

Nitrogen (N) is one of the most critical nutrients required by many field crops (Bender et al., 2015; Fan et al., 2019) such as soybean (*Glycine max* L.), which can accumulate up to 500 kg N ha^{-1} (Balboa et al., 2018; Ciampitti and Salvagiotti, 2018). Soil mineral N and biological N_2 fixation (BNF) are the main N sources for soybeans (Herridge et al., 2008; Peoples et al., 2009). The BNF process delivers the most sustainable N to support plant nutrient demand, reducing the reliance on soil N supply and the mining of the soil N reservoir (Crews and Peoples, 2005). However, under acidic soil conditions, such as low pH and high exchangeable aluminum (Al^{3+}), BNF can be impacted *via* a decrease in both rhizobium development and plant growth (Sartain and Kamprath, 1975; Alva et al., 1990; Evans et al., 1990).

All around the globe, acid soils represent 50% of the agricultural land (Von Uexküll and Mutert, 1995). In Brazil, it is estimated that 75% of the areas with potential for agricultural activity present problems related to soil acidity (Abreu et al., 2003; Fageria and Baligar, 2003). Additionally, more than 28 million hectares planted with soybeans in Brazil present issues related to soil acidity, hampering the crop attainable yield (CONAB, 2020). Soil acidity is usually characterized by low pH and often severe Al^{3+} toxicity (Sumner and Noble, 2003; Osman, 2018), affecting both root and shoot growth and overall soybean productivity (Ferguson et al., 2013; Fageria and Nascente, 2014; Kopittke et al., 2015; Ferguson and Gresshoff, 2015). In a greenhouse study, Lin et al. (2012) found that the number of soybean nodules drastically decreased

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3. Artigo publicado na *Applied Soil Ecology* referente ao quarto capítulo da presente tese.

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

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ABSTRACT

Integrated crop–livestock systems are recognized for intensifying food production as long as adequate management of grazing, fertilization, and liming is practiced. There is still a lack of information about the effect of these factors on the soil microbiological community. This study evaluated the effects of grazing, liming, and different P and K fertilization strategies on the soil microbial community and its relationship with soil chemical properties. For this purpose, a field experiment was established in 2017 on a subtropical Acrisol. Treatments consisted of two production systems: (i) integrated crop-livestock system (with winter sheep grazing) and (ii) specialized system (ungrazed), combined with two periods of P and K fertilization: (i) conventional fertilization (at soybean planting) and (ii) system fertilization (in the Italian ryegrass pasture). The effect of liming (with and without) was also evaluated. Eighteen months after lime application, the soil was sampled in the 0–5 and10–15 cm soil layers to evaluate soil chemical properties (pH, exchangeable Al, Ca, and Mg, available P and K, cation (Ca+Mg+K) saturation, and Al saturation) and soil microbial community composition. In the surface soil layer, a negative impact of lime application was observed for Gram+ bacteria (−29 %) and actinomycetes (−21 %), consequently decreasing the total bacterial community (−26 %) and total microbial biomass (−27 %). This negative impact of liming on Gram+ bacteria and actinomycetes was negatively related to the increase in soil pH, exchangeable Ca and Mg concentrations, and positively related to Al saturation and available K. The combination of an integrated system or system fertilization with lime increased arbuscular mycorrhizal fungi community 3.0-fold compared to a specialized system or conventional fertilization in the subsurface soil layer. In addition, sheep grazing increased saprophytic fungi biomass by 50 % in the subsurface soil layer compared to the specialized system. These positive impacts on the soil fungal community were probably associated with increased plant residue input. Sheep grazing and the fertilization strategy did not affect soil chemical properties or the microbial community at the soil surface. Liming, system fertilization, and sheep grazing benefitted the soil fungal community, thus improving soil health in highly weathered agricultural soils.

1. Introduction

Integrated crop-livestock systems (ICLS) are defined as systems where temporary rotation or alternating cash crops with pastures over time (Moraes et al., 2014) and are envisioned as routes for sustainable intensification with economic viability for food production (Russelle et al., 2007; Allen et al., 2007; Lemaire et al., 2014). The ICLS has several benefits as they ensure high crop yields, diversifying farmers' income while preserving natural resources, and contributing to the agricultural sector's climate and economic resilience (Peyraud et al., 2014; Peterson et al., 2020). In addition to sustainable intensification of production systems, ICLS is recognized for improving soil health

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Resumo biográfico

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