

Article

Microbial Activity of *Bacillus* spp. in Soil as a Function of Liming and Gypsum Application in Pasture

Eduardo E. S. Lopes ¹, Carlos S. Tiritan ¹, Patrick S. Silva ¹, Aurenívia Bonifácio ², Ademir S. F. Araujo ³ and Fabio F. Araujo ^{1,*}

¹ Faculty of Agronomy, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente 19067-175, SP, Brazil; eduardoeslopes@hotmail.com (E.E.S.L.); tiritan@unoeste.br (C.S.T.); patrick.nitrick@gmail.com (P.S.S.)

² Department of Soil Science, Universidade Federal do Ceará (UFC), Fortaleza 60020-181, CE, Brazil; aurenivia@ufc.br

³ Agricultural Sciences Centre, Universidade Federal do Piauí (UFPI), Teresina 64049-550, PI, Brazil; asfaruaj@yahoo.com.br

* Correspondence: fabio@unoeste.br

Abstract

This study hypothesized that the combination of limestone and gypsum could promote higher pasture yield and affect soil biological properties, such as microbial biomass and enzymatic activity, and alter the population of *Bacillus* within a pasture system with *U. brizantha*. A factorial design was employed with two treatments—without and with lime (0 and 1.6 ton ha^{−1})—and four gypsum doses (0, 0.5, 1.0, and 2.0 ton ha^{−1}) in experimental field plots. Six successive cuts were made in the pasture, and at the end, soil samples were analyzed for microbial biomass, enzymatic activity, and the putative *Bacillus* spp. community. The association of gypsum application with liming modified soil microbial activity attributes. It was found that only the application of gypsum reduced the microbial biomass and increased the concentration of putative *Bacillus* spp. in the soil, which correlated positively with increased crop yield, especially with the higher gypsum dose applied. The combined use of limestone and gypsum did not alter the microbial biomass and showed the best performance in increasing yield with the lowest dose of gypsum applied. In both cases evaluated, with and without the use of gypsum in conjunction with liming, it may be possible to achieve significant increases in biomass accumulation in pasture.

Keywords: soil liming; microbial biomass; pastures production

1. Introduction

Pastures are essential to animal production, with *Urochloa brizantha* being one of the most cultivated pasture species in tropical regions. An advantage of using *U. brizantha* is its adaptation to soils with low fertility due to its efficient root system, which explores the soil depth better [1]. However, roots need suitable conditions to grow deep, such as reduced soil acidity. Liming is recognized as the most effective practice to reduce soil acidity, neutralize toxic elements and increase the availability of nutrients to plants [2]. On the other hand, the incorporation of limestone into the soil depth needs plowing and harrowing, which alters the physical structure of the soil, reducing the porosity, size distribution, and stability of aggregates.

To overcome these negative effects of plowing and harrowing to soil, the application of gypsum has been used as an alternative to move the limestone depth and reduce the toxic effect of aluminum or reduced potential acidity (H+Al) [3]. Therefore, the combination



Academic Editor: Yi Zhou

Received: 15 June 2026

Revised: 28 July 2026

Accepted: 31 July 2026

Published: 4 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

of limestone and gypsum could decrease the soil acidity at depth without the need for plowing and harrowing [4]. In a field study in soil with minimum tillage, a positive effect of a combination of limestone and gypsum decreased the soil acidity at depth (0.20–0.60 m) [5]. Recently, it was observed that limestone and gypsum changed the chemical properties of the soil, mainly increasing the soil pH at 0–40 cm deep after 36 months [6].

Although the combination of limestone and gypsum has proven effective at reducing soil acidity at depth, little is known about the combined effect of these elements on soil biological properties. Within soil biological properties, soil microbial biomass (SMB) plays an essential role, including the decomposition of organic matter and nutrient cycling [7], contributing to increased soil fertility [8]. Enzymatic activity derived from SMB intensifies the degradation of organic matter in the soil [9], affecting the cycling of C, N, and P [10]. These important biological properties are significantly influenced by soil pH [11], while liming has been observed to positively impact soil microbial biomass (SMB) and its activity [12]. Extracellular enzymes, such as arylsulfatase and glucosidase, can accumulate in the soil and reflect the cultivation history, while dehydrogenase, as an intracellular enzyme, detects microbial activity in real time [13–15]. Enzymatic activity can indicate nutritional stress and water deficit during crop management [16]. However, there is still a significant gap in understanding the effect of the combined use of liming and gypsum on these biological properties, particularly in the *Bacillus* spp. community, which is considered an important bacterial genus for pastures due to its plant-growth-promoting characteristic [17]. The genus *Bacillus* plays an important functional role in soil and in the rhizosphere of pastures because it includes species capable of promoting plant growth through multiple mechanisms, such as phytohormone production, phosphorus solubilization, siderophore synthesis, antimicrobial compound production, biofilm formation, and increased plant tolerance to abiotic stresses [18,19]. *Bacillus* has been highlighted as a rhizosphere group that contributes to promoting plant growth [20], and its presence may be indicated as helpful for plants when cultivated under abiotic stress conditions [17].

In this study, we hypothesized that the combination of limestone and gypsum could promote higher pasture yield and affect soil biological properties, such as SMB and enzymatic activity, and alter the population of *Bacillus* within a pasture system with *U. brizantha*. Thus, we evaluated the combination of limestone and different doses of gypsum on the pasture yield, SMB, enzymatic activity, and community of *Bacillus* spp. in the rhizosphere of *Urochloa brizantha* in sandy soil with low fertility.

2. Materials and Methods

The experiment was conducted in a pasture field with *Urochloa brizantha* (cv. Piatã) established in 2017 at Mirante do Paranapanema, Sao Paulo state, Brazil (22°29'29" S; 52°1'18" ET, 306 m above sea). The soil is classified as oxysol, with clay contents of 13% and 15% at 0–10 cm and 10–20 cm depths, respectively. The climate is tropical (Am), with average annual temperatures around 22.8 °C and a rainfall regime characterized by two distinct periods: a rainy one from October to March and a low-rainfall one from April to September. In October 2019, before the experiment, chemical analysis of the soil was carried out at 0–10 cm and 10–20 cm depths (Table 1). Based on the chemical analysis, the amount of limestone required to raise the base saturation to 80% for the two analyzed depths was determined, totaling 1.6 ton ha^{−1}. The experimental design used was randomized blocks in a 2 × 4 factorial scheme, with two treatments with limestone (0 and 1.6 ton ha^{−1}) and four treatments with gypsum (0, 0.5, 1.0, and 2.0 ton ha^{−1}), and four replications, which totalized 32 experimental plots (40 m²). The surface application of limestone and gypsum within plots occurred in December 2019.

Table 1. Chemical properties of the soil.

	pH	SOM	P	K	S	H+Al	Ca	Mg	BS
		g kg ⁻¹	----- mg kg ⁻¹ -----			----- mmolc kg ⁻¹ -----			%
0–10 cm	5.8	18.6	11	15.6	2.4	13.2	13	9.3	63
10–20 cm	5.4	13.1	3.6	12.1	2.2	14.8	9.4	6.4	52

SOM—soil organic matter; P—phosphorus; K—potassium; S—sulfur; H+Al—potential acidity; Ca—calcium; Mg—magnesium; BS—base saturation.

From March 2020, six grass cuts were carried out throughout the study at 90-day intervals. Grass samples were taken from 1 m² of each plot, cut at a height of 20 cm from the ground. They were then placed in a forced-air ventilation oven for mowing and subsequent weighing to determine the dry matter of the pasture. In May 2021, soil samples within each plot were collected at a depth of 0–20 cm, following the criterion of collecting 10 soil sub-samples per plot, which were combined to produce that plot's sample. The samples were immediately transferred to the laboratory and stored under refrigeration until analysis. Subsequently, microbial biomass C (MBC) and N (MBN) were assessed using the microwave method [21,22]. Basal respiration was assessed by titration of NaOH with HCl [23]. In addition, we assessed the activities of fluorescein diacetate hydrolysis (FDA) [24], dehydrogenase, *B*-glucosidase, and arylsulfatase [25]. Rhizosphere soil samples were obtained from five plants collected from each plot, using aliquots of soil loosely adhered to the roots. The evaluation of *Bacillus* spp. was carried out by the serial dilution method and plate counting, using tubes that were subjected to a thermal shock (70 °C for 20 min) in the first stage of dilution, with the aim to select spore-forming bacteria. The plates were incubated in an oven at 32 °C for 72 h, where the colonies formed were counted and morphologically and physiologically characterized as putative *Bacillus* isolates using the guide described in the Bergey's Manual of Determinative Bacteriology [26], and the final value was expressed as colony-forming units (CFU) per gram of soil.

The results obtained were subjected to analysis of variance (ANOVA) preceded by the F test at the 5% probability level. Means were compared using the least significant difference (LSD) test at the 5% probability level. All statistical analyses were performed using RStudio 1.4.1106 (ExpDes.pt and ggcorrplot packages).

3. Results

The results showed that without liming, the application of the highest dose of gypsum (2.0 ton ha⁻¹) was necessary to achieve a higher accumulated pasture biomass (~32 ton ha⁻¹) (Figure 1). However, when liming was applied to the soil, it was possible to get a high accumulated pasture biomass (~30 Mg ha⁻¹) with the application of the lowest dose of gypsum (0.5 ton ha⁻¹). When each pasture cut was analyzed, the application of gypsum with and without liming promoted a distinct response in pasture biomass production. The increase in gypsum rates, with and without liming, led to a linear increase in pasture biomass after the third and second cuts, respectively. However, a significant linear increase was observed up to the sixth pasture cut with the highest dose of gypsum (2.0 ton ha⁻¹).

The application of the highest dose of gypsum (2.0 ton ha⁻¹) decreased microbial biomass C and increased biomass N (Figure 2). Compared with the control, the application of gypsum without liming significantly decreased the content of microbial biomass C, while it did not vary microbial biomass C when liming was applied. The application of gypsum increased (at 1.0 ton ha⁻¹) and decreased (in all doses) the microbial C:N ratio, with and without liming, respectively. The highest dose of gypsum promoted an increase in the

concentration of *Bacillus* in the soil compared with the control, an effect that was most significant without liming.

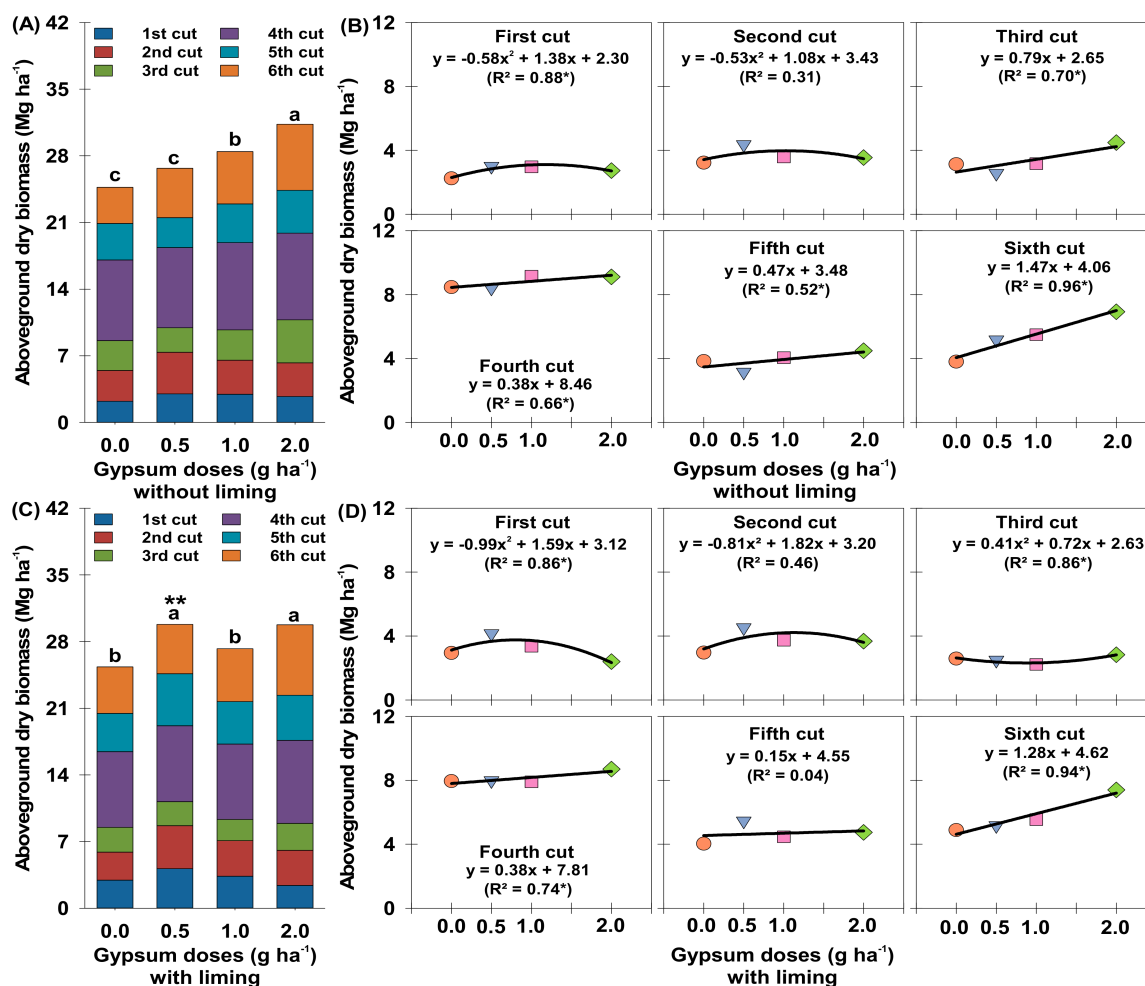


Figure 1. Production of shoot biomass accumulated (A,C) and after each cut (B,D) in response to gypsum doses combined or not with liming. In (A,C), different letters indicate significant differences between gypsum doses in the absence (–) or presence (+) of liming, while double asterisks (**) indicate significant differences between the absence (–) and presence (+) of liming. In (B,D), a single asterisk (*) indicates that R² was significant at 5%.

The application of gypsum without liming did not affect the activities of FDA, B-glucosidase, or arylsulfatase (Figure 3). However, the activities of these enzymes increased compared with the control, with the lowest dose of gypsum (0.5 ton ha⁻¹) associated with liming. In contrast, dehydrogenase increased with the applications of 0.5 and 1.0 ton ha⁻¹ gypsum without liming, while it did not vary when liming was used.

Through correlation analysis of microbial attributes with gypsum doses and pasture yield, it was found that in the absence of liming, there was a positive correlation between the concentration of putative *Bacillus* spp. and gypsum doses, along with an increase in plant yield. Conversely, a negative interaction was observed with microbial biomass C under this condition (Figure 4). On the other hand, when liming was associated with gypsum doses, a positive correlation was observed between microbial biomass N and gypsum doses, as well as pasture yield.

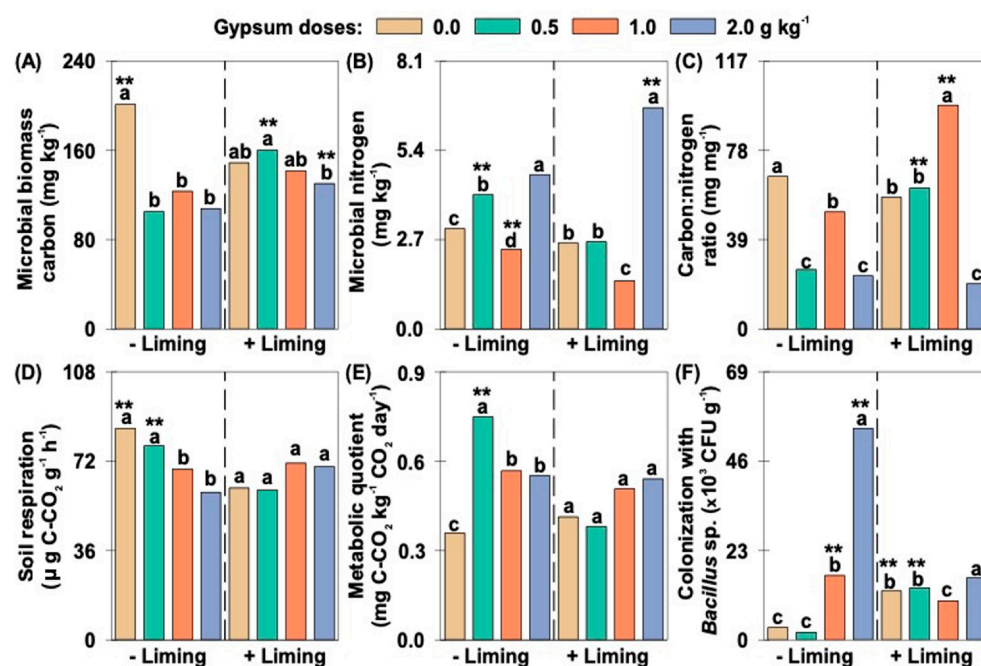


Figure 2. Microbial biomass C (A) and N (B), C:N ratio (C), basal respiration (D), metabolic quotient (E) and rhizosphere colonization with putative *Bacillus* spp. (F) in response to different gypsum doses in the absence (–) or presence (+) of liming. Different letters indicate significant differences between gypsum doses in the absence (–) or presence (+) of liming, while double asterisks (**) indicate significant differences between the absence (–) and presence (+) of liming.

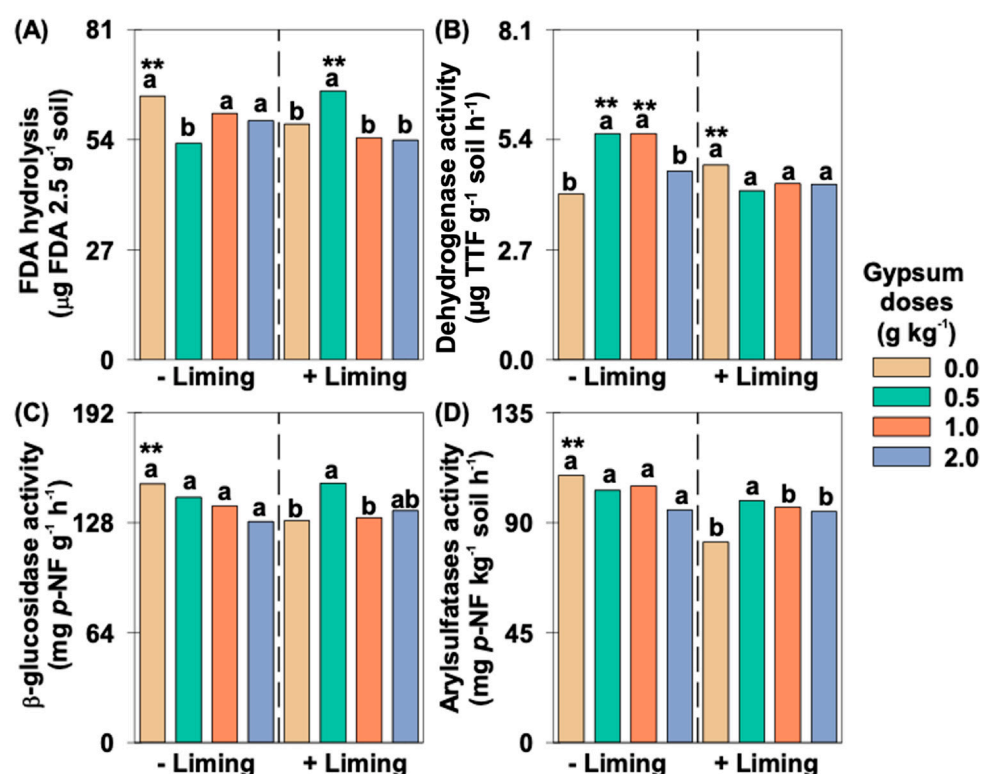


Figure 3. FDA hydrolysis (A), dehydrogenase activity (B), β-glucosidase activity (C), Arylsulfatase activity (D), in response to different gypsum doses in the absence (–) or presence (+) of liming. Different letters indicate significant differences between gypsum doses in the absence (–) or presence (+) of liming, while double asterisks (**) indicate significant differences between the absence (–) and presence (+) of liming.

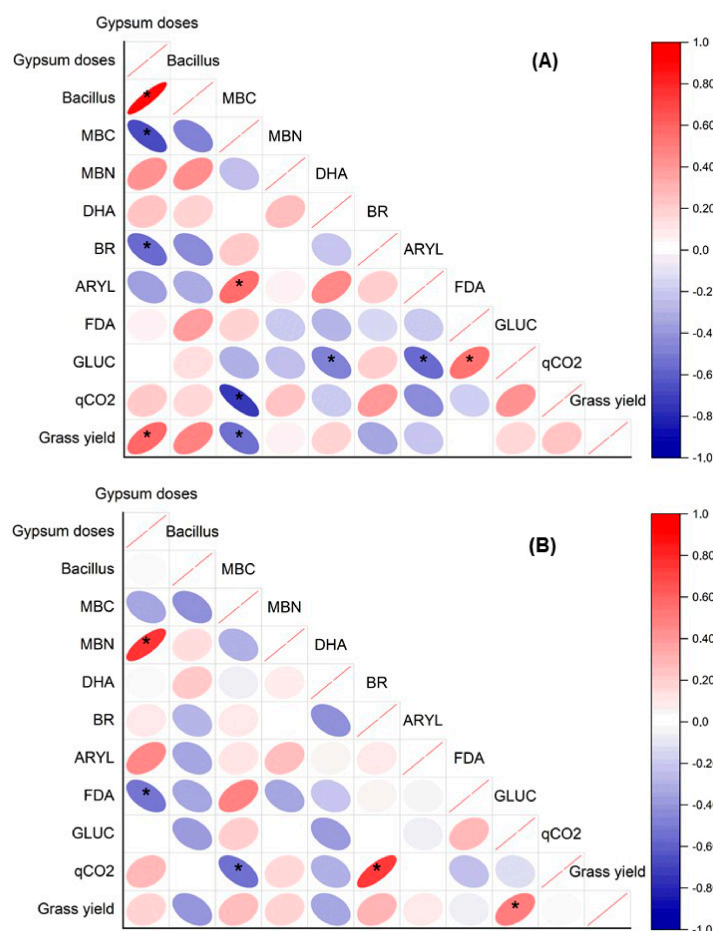


Figure 4. Pearson correlations of biological properties and pasture yield as a function of gypsum without (A) and with (B) limestone application. * indicates significant difference ($p < 0.05$).

4. Discussion

In line with the hypothesis, the combination of limestone and gypsum increased plant biomass and influenced soil biological properties, including soil microbial biomass and enzymatic activity, while also changing the abundance of putative *Bacillus* spp. within *Urochloa brizantha* pasture systems. The results showed that the highest accumulation of pasture biomass occurred with the application of lime + gypsum, making this combination an essential tool for food production in NT tropical agriculture, as was also reported in another study with an integrated system with pasture [27]. This increased biomass was due to the improved soil conditions facilitated by gypsum, such as higher soil pH and nutrient availability, specifically Ca^{2+} and S in the subsurface, which reduced Al^{3+} availability [28].

Furthermore, the application of the highest dose of gypsum without liming provided greater pasture biomass production in the sum of the six cuts performed, showing a greater residual effect. This finding reinforces previous studies that show persistent effects of gypsum for years and months after application [29,30]. In the condition when limestone was applied, this greater pasture biomass production was observed with the minimum dose of gypsum (0.5 tons ha^{-1}), suggesting synergism with this dose and the application of liming that may be useful for recommending correctives that provide favorable soil conditions for grass cultivation. This includes a better balance of bases in the soil, thus improving nutrient availability and increasing pasture biomass, even with lower doses of gypsum [27].

In the case of gypsum application without limestone, there was a reduction in soil microbial biomass carbon evaluated at the end of the study. The reduction in this biological

attribute has been associated, in many cases, with the search for carbon as an energy source, where fast-growing microorganisms, such as heterotrophic bacteria, can use part of the total microbial biomass as a nutrient source, with fungal hyphae as their preferred food [31]. The increase in pH and increased N mineralization of organic matter can modify the energy metabolism of the microbial community. Thus, the proportional increase in N in microbial biomass relative to C may indicate a microbial biomass with a lower C:N ratio, resulting in greater temporary immobilization of mineral N in the biomass [32]. Thus, a large part of the dead or dormant microbial biomass [15] may have been rapidly recycled as necromass, serving as a source of carbon and nutrients for the active community [33,34]. This impact may be associated with stimuli for increased microbial turnover, in which the community exhibits greater cell renewal, greater respiration, and greater use of labile substrates, without necessarily accumulating microbial biomass in the soil [33,35]. In other words, a restructuring of the microbial community may have occurred in the absence of liming and gypsum application, with this study verifying an increase in the metabolic quotient and a change in the carbon-to-nitrogen ratio in the biomass under the condition of absence of liming. On the other hand, the combination of gypsum and limestone did not cause significant changes in microbial biomass and also resulted in an increase in nitrogen (N) in the soil microbial biomass, highlighting the importance of the combination of gypsum and liming in soil microbial dynamics and nutrient cycling processes, such as mineralization and immobilization.

The increased availability of root exudates, resulting from good pasture development, can also favor fast-growing bacterial groups capable of rapidly exploiting readily available carbon sources in the rhizosphere [36,37]. In this context, the increase in the putative *Bacillus* spp. community in treatments with higher doses of gypsum may indicate a favoring of copiotrophic and metabolically active bacteria, which efficiently utilize labile organic compounds, exudates, and microbial residues. This is consistent with a previous study on the application of gypsum in sandy soil cultivated with rice, which observed an increase in *Bacillus* spp. [38]. Regarding the increase in putative *Bacillus* spp. in the absence of liming, it has also been reported that the presence of *Bacillus* spp. in the rhizosphere of tropical pastures showed a negative interaction with the concentration of calcium in the soil [39].

The association of gypsum with liming has been reported as a positive effect for increasing glucosidase and arylsulfatase activities under conditions of lower applied doses, especially in the long term [40,41]. In our study, this effect was observed in all extracellular enzymes evaluated, but only under the condition of liming associated with a lower dose of gypsum. The increase in enzymatic activity in the liming and gypsum treatments can be attributed to the improvement of soil chemical conditions, especially the increase in pH, greater availability of Ca^{2+} and Mg^{2+} , and reduction in Al^{3+} toxicity. These changes favor root growth and microbial activity, increasing the production of enzymes involved in the C, N, P, and S cycles, favoring bacterial groups over fungi, which have a lower C:N ratio [40]. On the other hand, dehydrogenase was not influenced by any dose of gypsum associated with liming. Calcium availability is directly related to the organic C cycle in the soil, assisting microbial metabolism in the presence of gypsum [42]. In the specific case of dehydrogenase activity, an increase in its activity occurred only under the condition without liming. This enzyme reflects the current oxidative potential of the microbial community, which is essential for mineralization processes that make nutrients available to plants [16,43].

Bacillus species are well-adapted to soil, with higher temperatures and low nutrient availability, and their ability to form spores increases their resilience to adverse environmental conditions commonly found in pasture soils [44]. They are classified as plant growth promoters and are present in several biological inputs on the market, and thus, are con-

sidered of great interest to agriculture [45,46]. This bacterial genus is of great importance to pastures due to its plant-growth-promoting characteristic [17]. This study found that under the condition of applying the highest doses of gypsum without liming, there was an increase in the concentration of putative *Bacillus* spp., which showed a positive correlation with pasture yield.

This study found that applying gypsum in the absence of liming reduces the carbon content of soil microbial biomass and increases the metabolic quotient. Furthermore, at the maximum gypsum dose, a higher concentration of putative *Bacillus* spp. was found in the soil, along with an increase in pasture biomass production. Liming combined with gypsum application showed that with the lowest gypsum dose, there was greater plant biomass production and a better balance of microbial biomass and enzymatic activity in the soil. More precise use of soil amendments can avoid economic waste in practice, preventing excessive doses without increasing crop yield. The promising data found in this study encourage further research under different edaphoclimatic conditions, as well as the confirmation of putative *Bacillus* spp. using the 16S rDNA gene.

5. Conclusions

The association of gypsum application with liming modified soil microbial activity attributes. It was found that only the application of gypsum reduced microbial biomass and increased the abundance of putative *Bacillus* spp. in the soil, which correlated positively with increased crop yield, especially with the highest dose of gypsum applied. The combined use of limestone and gypsum did not alter microbial biomass and had the best performance regarding increasing yield with the lowest dose of gypsum applied. In both cases, it may be possible to achieve high levels of biomass accumulation in pasture.

The results reinforce the importance of managing the soil profile under pastures with a greater supply of bases to the soil in two different ways, resulting in improved microbial activity and system yield.

Author Contributions: Conceptualization, C.S.T. and F.F.A.; methodology, F.F.A.; software, A.B.; validation, P.S.S., A.S.F.A. and E.E.S.L.; formal analysis, A.S.F.A.; investigation, E.E.S.L.; resources, F.F.A.; data curation, E.E.S.L.; writing—original draft preparation, F.F.A.; writing—review and editing, A.S.F.A.; visualization, A.B.; supervision, A.S.F.A.; project administration, C.S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The datasets presented in this article are not readily available because the data are part of an ongoing study. Requests to access the datasets should be directed to [Fabio F. Araujo—fabio@unoeste.br].

Acknowledgments: Fabio F. Araujo and Ademir S.F. Araujo are fellow of CNPq.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Pezzopane, C.G.; Santos, P.M.; Cruz, P.G.D.; Altoé, J.; Ribeiro, F.A.; Valle, C.B.D. Hydric deficiency in genotypes of *Brachiaria brizantha*. *Ciênc. Rural* **2015**, *45*, 871–876. [\[CrossRef\]](#)
2. Mahmud, M.S.; Chong, K.P. Effects of liming on soil properties and its roles in increasing the productivity and profitability of the oil palm industry in Malaysia. *Agriculture* **2022**, *12*, 322. [\[CrossRef\]](#)
3. Anderson, G.C.; Pathan, S.; Hall, D.J.M.; Sharma, R.; Easton, J. Short- and long-term effects of lime and gypsum applications on acid soils in a water-limited environment: 3. Soil solution chemistry. *Agronomy* **2021**, *11*, 826. [\[CrossRef\]](#)
4. Li, G.D.; Conyers, M.K.; Helyar, K.R.; Lisle, C.J.; Poile, G.J.; Cullis, B.R. Long-term surface application of lime ameliorates subsurface soil acidity in the mixed farming zone of south-eastern australia. *Geoderma* **2019**, *338*, 236–246. [\[CrossRef\]](#)

5. Soratto, R.P.; Crusciol, C.A.C. Atributos químicos do solo decorrentes da aplicação em superfície de calcário e gesso em sistema de plantio direto recém- implantado. *Rev. Bras. Cienc. Solo* **2008**, *32*, 675–688. [\[CrossRef\]](#)
6. Minato, E.A.; Brignoli, F.M.; Neto, M.E.; Besen, M.R.; Cassim, B.M.A.R.; Lima, R.S.; Tormena, C.A.; Inoue, T.T.; Batista, M.A. Lime and gypsum application to low-acidity soils: Changes in soil chemical properties, residual lime content and crop agronomic performance. *Soil Tillage Res.* **2023**, *234*, 105860. [\[CrossRef\]](#)
7. Gupta, A.; Singh, U.B.; Sahu, P.K.; Paul, S.; Kumar, A.; Malviya, D.; Singh, S.; Kuppusamy, P.; Singh, P.; Paul, D.; et al. Linking soil microbial diversity to modern agriculture practices: A review. *Int. J. Environ. Res. Public Health* **2022**, *7*, 3141. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Piotrowska-Długosz, A.; Długosz, J.; Gryta, A.; Frac, M. Responses of n-cycling enzyme activities and functional diversity of soil microorganisms to soil depth, pedogenic processes and cultivated plants. *Agronomy* **2022**, *12*, 264. [\[CrossRef\]](#)
9. Ayala-montejo, D.; Valdés-Velarde, E.; Benedicto-Valdés, G.S.; Escamilla-Prado, E.; Sánchez-Hernández, R.; Gallardo, J.F.; Martínez-Zurimendi, P. Soil biological activity, carbon and nitrogen dynamics in modified coffee agroforestry systems in mexico. *Agronomy* **2022**, *12*, 1794. [\[CrossRef\]](#)
10. de Melo, W.J.; de Melo, G.M.P.; de Melo, V.P.; Araujo, A.S.F.; Ferraudo, A.S.; Bertipaglia, L.M.A. Soil microbial biomass and enzyme activity in six brazilian oxisols under cropland and native vegetation. *Bragantia* **2020**, *79*, 623–629. [\[CrossRef\]](#)
11. Wang, X.; Xu, C.; Jaroniec, M.; Zheng, Y.; Qiao, S.-Z. Anomalous hydrogen evolution behavior in high-ph environment induced by locally generated hydronium ions. *Nat. Commun.* **2019**, *10*, 4876. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Holland, J.; Bennett, A.; Newton, A.; White, P.; McKenzie, B.; George, T.; Pakeman, R.; Bailey, J.; Fornara, D.; Hayes, R. Liming impacts on soils, crops and biodiversity in the uk: A review. *Sci. Total Environ.* **2018**, *610–611*, 316–332. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Dou, Z.; Ma, J.; Wang, K.; Lu, Q.; Chi, Z.; Cui, D.; Pan, C.; He, Z.; Zuo, Y. Use of soil nematodes as indicators of soil and plant health in continuous cropping systems: A case study in dragon fruit. *J. Integr. Agric.* **2025**, *24*, 1987–2001. [\[CrossRef\]](#)
14. Razavi, B.S.; Hoang, D.T.; Blagodatskaya, E.; Kuzyakov, Y. Mapping the footprint of nematodes in the rhizosphere: Cluster root formation and spatial distribution of enzyme activities. *Soil Biol. Biochem.* **2017**, *115*, 213–220. [\[CrossRef\]](#)
15. Blagodatskaya, E.; Kuzyakov, Y. Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biol. Biochem.* **2013**, *67*, 192–211. [\[CrossRef\]](#)
16. Meena, S.N.; Sharma, S.K.; Singh, P.; Ram, A.; Meena, B.P.; Jain, D.; Singh, D.; Debnath, S.; Yadav, S.; Dhakad, U.; et al. Tillage-based nutrient management practices for sustaining productivity and soil health in the soybean–wheat cropping system in vertisols of the indian semi-arid tropics. *Front. Sustain. Food Syst.* **2023**, *7*, 1234344. [\[CrossRef\]](#)
17. Mukhtar, S.; Mirza, M.S.; Awan, H.A.; Asma Maqbool, A.M.; Samina Mehnaz, S.M.; Malik, K.A. Microbial diversity and metagenomic analysis of the rhizosphere of para grass (*Urochloa mutica*) growing under saline conditions. *Pak. J. Bot.* **2016**, *48*, 779–791.
18. Hayat, R.; Ali, S.; Amara, U.; Khalid, R.; Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.* **2010**, *60*, 579–598. [\[CrossRef\]](#)
19. Radhakrishnan, R.; Hashem, A.; Abd_Allah, E.F. *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Front. Physiol.* **2017**, *8*, 667. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Mutai, C.; Njuguna, J.; Ghimire, S. *Brachiaria* Grasses (*Brachiaria* spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. *Microbiol. Open* **2017**, *6*, e00497. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass carbon. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [\[CrossRef\]](#)
22. Brookes, P.C.; Kragt, J.; Powlson, D.; Jenkinson, D. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *6*, 831. [\[CrossRef\]](#)
23. Jenkinson, D.S.; Powlson, D.S. The effects of biocidal treatments on metabolism in soil-I. Fumigation with chloroform. *Soil Biol. Biochem.* **1976**, *8*, 167–177. [\[CrossRef\]](#)
24. Schnürer, J.; Rosswall, T. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* **1982**, *43*, 1256–1261. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Tabatabai, M.A. Soil enzymes. In *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties*; Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A., Eds.; SSSA: Madison, WI, USA, 1994; pp. 775–833.
26. Li, D.; Alexander, M. Co-inoculation with antibiotic producing bacteria to increase colonization and nodulation by rhizobia. *Plant Soil* **1988**, *108*, 211–219. [\[CrossRef\]](#)
27. Crusciol, C.A.C.; Marques, R.R.; Carmeis Filho, A.C.A.; Soratto, R.P.; Costa, C.H.M.; Neto, J.F.; Castro, G.S.A.; Pariz, C.M.; Castilhos, A.M.; Franzluebbers, A.J. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. *Nutr. Cycl. Agroecosyst.* **2019**, *115*, 347–372. [\[CrossRef\]](#)
28. Besen, M.R.; Ribeiro, R.H.; Esper, M.; Minato, E.A.; Coneglian, C.F.; Kachinski, W.D.; Tormena, C.A.; Inoue, T.T.; Batista, M.A. Lime and phosphogypsum application management: Changes in soil acidity, sulfur availability and crop yield. *Rev. Bras. Cienc. Solo* **2021**, *45*, e0200135. [\[CrossRef\]](#)

29. Pauletti, V.; de Pierri, L.; Ranzan, T.; Barth, G.; Motta, A.C.V. Efeitos em longo prazo da aplicação de gesso e calcário no sistema de plantio direto. *Rev. Bras. Ciênc. Solo* **2014**, *38*, 495–505. [[CrossRef](#)]
30. Araújo, L.G.; de Sousa, D.M.G.; de Figueiredo, C.C.; Rein, T.A.; Nunes, R.d.S.; Júnior, J.d.D.G.d.S. The residual effect of gypsum on subsoil conditioning, nutrition and productivity of sugarcane crops. *Aust. J. Crop Sci.* **2018**, *12*, 1313–1321. [[CrossRef](#)]
31. Brabcová, V.; Štursová, M.; Baldrian, P. Nutrient content affects the turnover of fungal biomass in forest topsoil and the composition of associated microbial communities. *Soil Biol. Biochem.* **2018**, *118*, 187–198. [[CrossRef](#)]
32. Malik, A.A.; Chowdhury, S.; Schlager, V.; Oliver, A.; Puissant, J.; Vazquez, P.G.M.; Jehmlich, N.; von Bergen, M.; Griffiths, R.I.; Gleixner, G. Soil Fungal:Bacterial Ratios Are Linked to Altered Carbon Cycling. *Front. Microbiol.* **2016**, *7*, 1247. [[CrossRef](#)] [[PubMed](#)]
33. Kastner, M.; Miltner, A.; Thiele-bruhn, S.; Liang, C. Microbial necromass in soils-linking microbes to soil processes and carbon turnover. *Front. Environ. Sci.* **2021**, *9*, 756378. [[CrossRef](#)]
34. Cantoran, A.; Maillard, F.; Baldrian, P.; Kennedy, P.G. Defining a core microbial necrobiome associated with decomposing fungal necromass. *FEMS Microbiol. Ecol.* **2023**, *99*, 98. [[CrossRef](#)] [[PubMed](#)]
35. Buckeridge, K.M.; Creamer, C.; Whitaker, J. Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Funct. Ecol.* **2022**, *36*, 1396–1410. [[CrossRef](#)]
36. Eisenhauer, N.; Lanoue, A.; Strecker, T.; Scheu, S.; Steinauer, K.; Thakur, M.P.; Mommer, L. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci. Rep.* **2017**, *7*, e44641. [[CrossRef](#)] [[PubMed](#)]
37. Ma, W.; Tang, S.; Dengzeng, Z.; Zhang, D.; Zhang, T.; Ma, X. Root exudates contribute to belowground ecosystem hotspots: A review. *Front. Microbiol.* **2022**, *13*, 937940. [[CrossRef](#)] [[PubMed](#)]
38. Rocha, G.T.; Montalvão, S.C.L.; Queiroz, P.R.M.; Berçot, M.R.; Gomes, A.C.M.M.; Monnerat, R.G. Morphological and biochemical characterization of bacterial species of *Bacillus*, *Lysinibacillus* and *Brevibacillus*. *Rev. Ceres* **2023**, *91*, 104. [[CrossRef](#)]
39. Araújo, F.F.; Pedroso, R.A.B. Interação de *Bacillus* sp. Com a rizosfera de três espécies de plantas forrageiras. *Biosci. J.* **2013**, *29*, 152–158.
40. Inagaki, T.M.; Sá, J.C.d.M.; Caires, E.F.; Gonçalves, D.R.P. Lime and gypsum application increases biological activity, carbon pools, and agronomic productivity in highly weathered soil. *Agric. Ecosyst. Environ.* **2016**, *231*, 156–165. [[CrossRef](#)]
41. Luiz, M.D.S.; Junior, L.A.Z.; Ribeiro, M.R.; de Matos, M.A.; Andrade, D.S. Residual effects of agricultural gypsum on soil chemical and microbiological characteristics. *Soil Use Manag.* **2022**, *38*, 1656–1666. [[CrossRef](#)]
42. Shabtai, I.A.; Wilhelm, R.C.; Schweizer, S.A.; Höschen, C.; Buckley, D.H.; Lehmann, J. Calcium promotes persistent soil organic matter by altering microbial transformation of plant litter. *Nat. Commun.* **2023**, *14*, 6609. [[CrossRef](#)] [[PubMed](#)]
43. Jat, H.S.; Choudhary, M.; Datta, A.; Yadav, A.K.; Meena, M.D.; Devi, R.; Gathala, M.K.; Jat, M.L.; McDonald, A.; Sharma, P.C. Temporal changes in soil microbial properties and nutrient dynamics under climate smart agriculture practices. *Soil Tillage Res.* **2020**, *199*, 104595. [[CrossRef](#)] [[PubMed](#)]
44. Rampelotto, P.H.; Ferreira, A.d.S.; Barboza, A.D.M.; Roesch, L.F.W. Changes in diversity, abundance, and structure of soil bacterial communities in brazilian savanna under different land use systems. *Microb. Ecol.* **2013**, *66*, 593–607. [[CrossRef](#)] [[PubMed](#)]
45. Mendis, H.C.; Thomas, V.P.; Schwientek, P.; Salamzade, R.; Chien, J.T.; Waidyarathne, P.; Kloepper, J.; De La Fuente, L. Strain-specific quantification of root colonization by plant growth promoting rhizobacteria *Bacillus firmus* i-1582 and *Bacillus amyloliquefaciens* qst713 in non-sterile soil and field conditions. *PLoS ONE* **2018**, *13*, e0193119. [[CrossRef](#)] [[PubMed](#)]
46. Figueiredo, M.V.B.; Rodrigues, A.B.; Cerqueira, A.; Araujo, F.F. Plant growth promoting rhizobacteria: Key mechanisms of action. In *Microbial-Mediated Induced Systemic Resistance in Plants*; Springer: Singapore, 2016; pp. 23–37.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.