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RESEARCH ARTICLE

# Soil health response to soil biological conditioner in Brazilian soybean fields

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## Abstract

Biological products used in soybean seed treatment can enhance soil microbial activity, thereby improving soil health. Brazil is the world's largest producer of soybeans and has a vast and diverse cultivation area characterized by varying weather and soil conditions. However, there is a lack of studies that have assessed the soil health response to soil biological conditioners based on calcium sulfate dihydrate applied by seed treatment at large-scale farmer-led and over extended periods in Brazilian soybean fields. To address this gap, we carried out a large-scale farmer-led study across a 3000-km transect to evaluate the soil health responses to a biological conditioner over three consecutive years. Soil health indicators including soil organic carbon, extracellular ß-glucosidase enzyme activity, soil bulk density, soil pH, available phosphorus, and exchangeable potassium were measured, interpreted, and integrated into a soil health index (SMAF-SHI) to compare experimental strips with and without of the soil biological conditioner. A dataset of 87 sampling points collected from 15 farmer-led experiments over three consecutive years of the soil biological conditioner application (i.e., 2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) was analyzed. The results showed site- and year-specific alterations on soil chemical, physical, and biological indicators, as well as overall SMAF-SHI. In general, the effects of the soil biological conditioner application were subtle and statistically undetectable for most of the metrics over three consecutive years of application. However, we observed potential changes in soil organic carbon, extracellular β-glucosidase enzyme activity, and soil bulk density indicators after two and three years of the soil biological conditioner application. To further understand the long-term effects of biological conditioners on soil, we propose continued soil health monitoring over time, with a particular focus on the rhizosphere, and the inclusion of molecular biology methods to measure the abundance, diversity and functionality of the soil microbiome.

**Keywords:** Biostimulants; sustainable agriculture; soil quality indicators; soil quality; calcium sulfate dehydrate; seed dressing

## Introduction

In the last decade, there has been an extraordinary increase in the agricultural biological market. This market accounts for USD 9.9 billion worldwide (Grand View Research 2023) and has increased especially in Brazil (Soares *et al.*, 2023). This has been driven by the global tendency toward sustainable agricultural practices and the growing environmental concerns in agriculture. Soybean is a representative leguminous cash crop that accounts for about 42 million hectares in Brazil (IBGE 2024) and its cultivation is the most successful example of biological products application, mainly related to microbial inoculants (Santos *et al.* 2019). Using biological products could be a possible alternative to decrease environmental impacts and costs related to fertilizer use (Santos *et al.* 2024) and improve the yield throughout biological processes (Bender *et al.*, 2016; Elias *et al.*, 2024). In addition, the industry has developed biological products that can be used as seed treatments to promote soil microbial activity (Johnston-Monje *et al.*, 2016; Ahsan *et al.*, 2023). For instance, there is manufactured by-products building on limestone, dolomite, polymers (Babla *et al.* 2022), as well as calcium sulfate dehydrated (Maris *et al.* 2021; Wang *et al.* 2024). Another significant aspect of biological products is their potential to promote root growth and exudation, which in turn can enhance soil biological activity (Rasmann & Hiltpold 2022). Therefore, conservation agriculture practices associated with new biological products can improve soil health (Wadduwage *et al.* 2024) and, consequently, increase primary productivity (Romero *et al.* 2024) and resilience to climate change (Qiao *et al.* 2022).

Soil health is the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Lehmann *et al.* 2020). It is the foundation of nature's contribution to people such as plant production, water quality, climate regulation, and human health (Adhikari & Hartemink 2016). Soil health assessment encompasses the measurement of soil chemical, physical, and biological indicators (Bünemann *et al.* 2018) which represent the soil functions and ability of soils to provide ecosystem services. Fertilizers combined with different soil additives such as amino acids can improve soil health by increasing soil biological activity (Wang *et al.* 2024), which in turn enhances extracellular enzyme activity (Gómez *et al.* 2020) and soil organic carbon content (Trivedi *et al.* 2016). In addition, biological activity is linked to soil chemicals (Zwetsloot *et al.* 2022; Peixoto *et al.* 2010) and physical attributes (Creamer *et al.* 2022;Yudina & Kuzyakov 2023). While soil biological activity responds quickly to changes in the soil environment, the overall soil health response to biological conditioners should be assessed over a longer period than just a short time (e.g., one year). This variability has been attributed to factors such as weather conditions (Maris *et al.* 2021), soil types, regional climate variability, and time (Galluzzi *et al.* 2024). All of these influence the plant-soil-environment interactions as well as the stability and equilibrium of soil processes that require more than just one year to stabilize and impact soil health.

Therefore, soil conditioners can improve soil health through nutrient cycling, soil structure development, the activity of soil organisms and extracellular enzymes, and soil organic carbon content. Besides the growing of biological products used in Brazilian soybean cultivation, there is an absence of studies that elucidate the effects of soil biological conditioners on soil health (by integrating chemical, physical, and biological aspects) at large-scale farmer-led in the country. In addition, the assessment of soil health indicators is necessary for understanding how agricultural management strategies and environmental factors affect the biological, chemical, and physical relationships at the soil-rhizosphere-plant systems and their impact on sustainable agriculture in a long-term (Tahat *et al.* 2020).There is a global trend towards on-farm (e.g., Schulte *et al.* (2015), Williams *et al.* (2020), Krupek *et al.* (2022), van der Pol *et al.* (2022), Schiebelbein and Cherubin, 2024)) and large-scale (Wood & Bowman 2021) soil health assessments. Therefore, we carried out a large-scale farmer-led study to evaluate the 3-year soil health responses to a soil biological conditioner in Brazilian soybean fields. The study hypothesis was that a biological conditioner based on calcium sulfate dehydrated increases soil biological activity and consequently improves overall soil health across a 3000-km transect of the Brazilian soybean region.

## Material and methods

### *Study sites and experimental design*

To assess the impact of the soil biological conditioner on soil health, we analyzed a dataset consisting of 87 sampling points collected from 15 farmer-led experiments conducted between 2021 and 2023. The study encompasses data from seven Brazilian states (Figure 1) across a 3000-km transect in the

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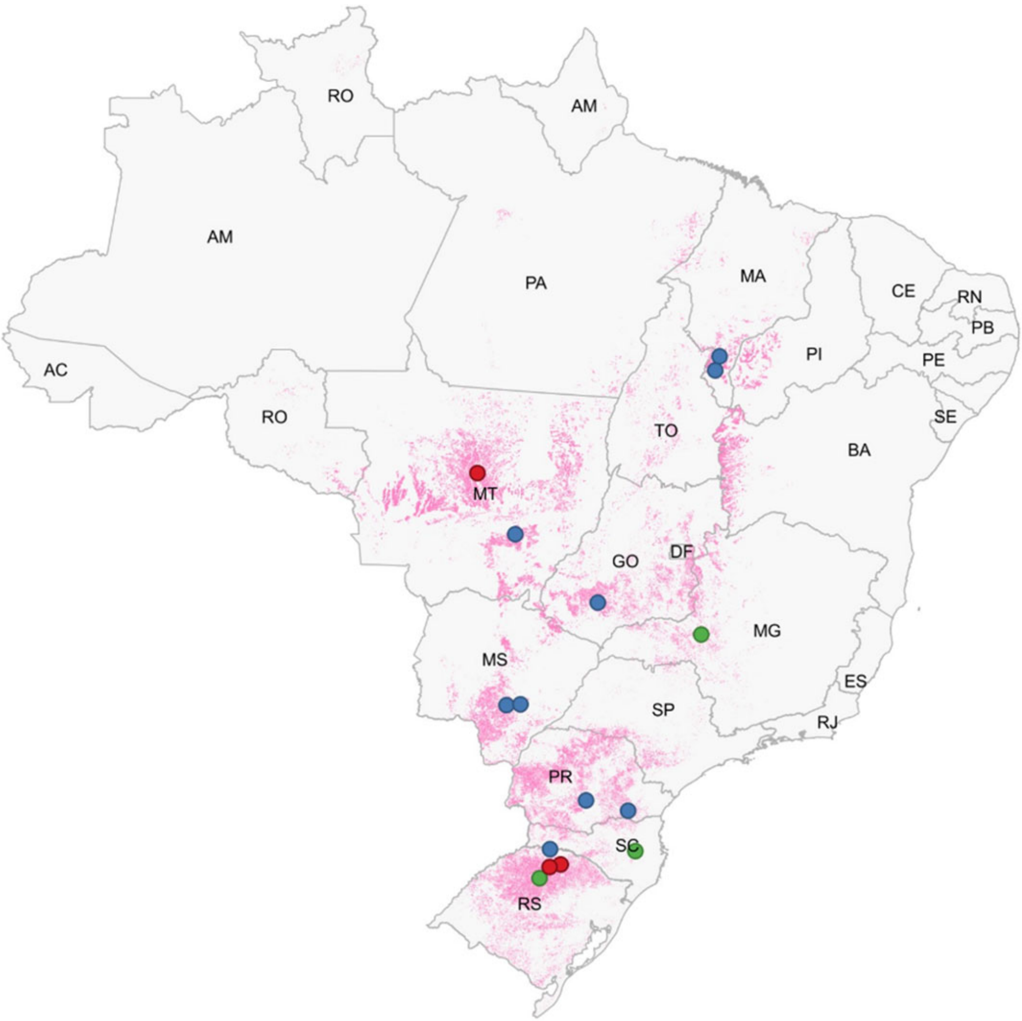


**Figure 1.** Distribution of soybean cultivation area (rose color) that accounts for 42 million hectares in Brazil and location of farms (points). The points are colored according to the years of the biological conditioner was applied: 2021 – correspond to one application (blue), 2022 – correspond to two applications (green), and 2023 – correspond to three applications (red). Source of soybean cultivation area: MAPBIOMAS (https://plataforma.brasil.mapbiomas.org/).

country‘s largest soybean-producing region. The soil classification, texture, and location of each farm are provided in Table 1. The experimental design was established to compare strips with biological conditioner application to strips without application (control). The trial consisted of 100 × 200 m strips in each farm. The soil biological conditioner consisted of calcium sulfate dihydrate with 35% sulfur dioxide ($SO_3$) and 25% calcium oxide (CaO). This is a commercially available biological conditioner (*BLUSOIA*®) from BluAgri Biotechnology Company®. The total amino acids present in the constitution of the product can be verified in Table S1. Amino acids play a role in plant-soil-microbiome interactions, stimulating biological activity around the roots (Compant *et al.* 2019) which can impact soil health over time besides the conditioner has no residual effect on soil. At each treatment strip, the soil biological conditioner was applied by seed treatment at the rate of 300 g $ha^{-1}$ each year according to the technical recommendation of BluAgri Company®. All farmers managed soil with no tillage and followed established fertilizer recommendations for soybean

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**Table 1.** Farm's location (Region, State, and municipality) and soil information (characterization and classification)

| Region | State | Municipality | Clay (%) | Silt (%) | Sand (%) | Soil texture | Brazilian Classification[1] | WRB- FAO[2] | Soil Taxonomy[3] |
|---|---|---|---|---|---|---|---|---|---|
| South | Rio Grande do Sul | Santa Bárbara do Sul | 28 | 10 | 62 | Sandy clay loam | LVw | Oxisols | Ferralsols |
| | | Quatro Irmãos | 56 | 30 | 10 | Clayey | LVd | Oxisols | Ferralsols |
| | | Sarandi | 58 | 26 | 16 | Clayey | LVdf | Oxisols | Ferralsols |
| | | Rio dos Índios | 34 | 36 | 30 | Clayey loam | LVdf | Oxisols | Ferralsols |
| | Santa Catarina | Aurora | 48 | 36 | 16 | Clayey | CXbd | Inceptisols | Cambisols |
| | Paraná | Lapa | 46 | 24 | 30 | Clayey | CXbd | Inceptisols | Cambisols |
| | | Guarapuava | 72 | 22 | 6 | Clayey | LBd | Oxisols | Ferralsols |
| Midwest | Mato Grosso do Sul | Rio Brilhante | 57 | 21 | 22 | Clayey | LVdf | Oxisols | Ferralsols |
| | | Rio Brilhante | 63 | 21 | 18 | Clayey | LVdf | Oxisols | Ferralsols |
| | Mato Grosso | Sorriso | 55 | 8 | 37 | Clayey | LVAd | Oxisols | Ferralsols |
| | | Primavera do Leste | 30 | 5 | 65 | Sandy clay loam | LVd | Oxisols | Ferralsols |
| | Goias | Rio Verde | 43 | 8 | 49 | Sandy clay | LVAd | Oxisols | Ferralsols |
| Southeast | Minas Gerais | Patrocínio | 53 | 27 | 20 | Clayey | LVd | Oxisols | Ferralsols |
| Northeast | Maranhão | Balsas | 13 | 5 | 82 | Loamy sand | LAd | Oxisols | Ferralsols |
| | | Balsas | 25 | 4 | 71 | Sandy clay loam | LAd | Oxisols | Ferralsols |

[1]Latossolo Amarelo (LA), Latossolo Vermelho-Amarelo (LVA), distrófico (d), distroférrico (df). Latossolo Vermelho Escuro (LVw), Cambissolo Álico Tb A moderado (CXbd) (SANTOS *et al.* 2018).
[2](WRB 2015),
[3](Soil Survey Staff 2014).

production in Brazil. In addition, the soybean variety and its disease and pest control were carried out according to specific technical recommendations for each region. Therefore, the soil biological conditioner application was the single difference in the strips.

### *Soil sampling and measured indicators*

Soil sampling was carried out after the soybean harvest (February to May) for three consecutive years of the soil biological conditioner application (i.e., 2021 corresponds to one application, 2022 to two applications, and 2023 to three applications). In each farm, strip trials with control and treatment areas were established, with four pseudo-replicates located 50 m apart. The use of strip trials and pseudo-replicates is a common procedure in ecological and agronomic studies on commercial farms (e.g., Wood and Bowman, 2021; Cherubin *et al.*, 2024). Undisturbed soil cores were collected using metallic rings of 5 cm × 5 cm (∼100 $cm^3$) to measure soil bulk density (BD). Other disturbed soil cores were taken to measure soil chemical (i.e., soil pH, available phosphorus - P, and exchangeable potassium - K) and biological indicators (i.e., soil organic carbon - SOC and extracellular β-glucosidase enzyme activity - ß-G). All samples were taken at 0–10 and 10–20 cm soil layers.

The undisturbed soil cores were oven-dried at 105 °C for 48 h and weighed to quantify the BD (i.e., dry soil mass divided by the ring volume) according to Grossman and Reinsch (2002). The disturbed soil cores were oven-dried under forced air circulation at 45 °C to reach constant weight and then passed through a 2 mm diameter sieve to measure soil chemical and biological indicators. A glass electrode was used upon stirring and standing of 10 ml of soil mixed with 10 ml of distilled water for measuring the soil pH. Available P, and exchangeable K were extracted by the resin method and measured by flame photometry and via spectrophotometry, respectively, according to van Raij *et al.* (2001). The SOC content was determined by dry combustion using a LECO CN628 Carbon Analyzer (Nelson & Sommers 1996). The activity of β-G was measured by incubating the soil (1 g) with ρ-nitrophenyl β-glucopyranoside substrate (pH 6.0) at 37 °C for 1 h and measuring the released ρ-nitrophenol via spectrophotometry (410 nm) according to Tabatabai (1994).

### *Soil health assessment*

Soil health was measured using the Soil Management Assessment Framework (SMAF) according to Andrews *et al.* (2004). The SMAF encompasses a three-step procedure: I) selecting a minimum dataset; II) interpreting measured indicators; and III) integrating indicators into an overall soil health index. In the first step, six soil indicators were selected: bulk density to represent soil physical indicators, soil pH, test P, and test K to represent soil chemical indicators, and SOC and ß-G to represent soil biological indicators. These indicators are the most frequently used for soil health assessment in the world (Bünemann *et al.* 2018) and also in Brazil (Simon *et al.* 2022).

In the second step procedure, each indicator was scored by transforming measured values into 0-1 scores using non-linear scoring curves in the SMAF spreadsheet. The scoring curves developed for each soil indicator were based on site-specific class factors including soil texture, weather conditions for the sampling area, soil mineralogy, slope, and analytical method used for test P (Andrews *et al.*, 2004; Wienhold *et al.*, 2009). The texture class (used for scoring BD, SOC, and ß-G) had a factor ranging from 4 (applied to clayey soils) to 1 (applied to sandy soils) depending on different places. The mineralogy class (used for scoring BD) had a factor of 3 (1:1 clay and Fe and Al oxides) in all soils. The climate class (used for scoring SOC and ß-G) had a factor of 1 (≥170-degree day and ≥550 mm mean annual rainfall) in all soils. The organic matter class (used for scoring SOC and ß-G) had a factor of 4 (low organic matter). The slope and weathering class (used for scoring test P) had a factor of 2 (2%–5% slope) and 2 (tropical soils) for weathering degree. The Resin method (class 5) was used for scoring test P (Cherubin *et al.* 2016). In addition,

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the thresholds of the soil pH, test P, and test K values followed the recommendations for soybean production in Brazil.

In the third step, all indicator scores were integrated into an overall soil health index (SHI) using a weighted additive approach (Eq. 1).

$$SHI = \sum SiWi \quad (1)$$

where Si is the indicator score and Wi is the weighted value of the indicators. The indicators were weighted based on physical (DB), biological (SOC and ß-G), and chemical (soil pH, test P, and test K) components. Each component had an equivalent weight (33.3%) in the final index, regardless of the number of indicators according to Cherubin *et al.* (2016).

### *Data analysis*

To assess the impact of soil conditioner on each soil health indicator and the soil health index, we calculated its effect on soil health indicators and SMAF-SHI for each year of application. This was done by calculating the 95% confidence interval, mean, upper, and lower values using Excel software, along with the 'broom' and 'meta' packages in R. We also generated graphs using the 'ggplot2' package to show the effect of the soil biological conditioner application. In addition, Tukey's test ($p < 0.05$) was performed for mean comparisons using data from the 3 out of 15 farms that applied the soil biological conditioner for three consecutive years (i.e., 2021, 2022, and 2023). All statistical procedures were conducted using R software (Core 2024).

## Results

An overview of measured values with mean values and standard deviation for each soil health indicator is presented in Table 2. The BD values ranged from 1.25 to 1.57 Mg m$^{-3}$ and 1.20 to 1.54 Mg m$^{-3}$ under control and the biological conditioner application (treatment), respectively. There was no effect of treatment after one (2021), two (2022), and three (2023) years of application on BD in both 0–10 (Figure 2a) and 10–20 cm (Figure 2d) soil layers, besides the amplitude of BD values. Therefore, the use of the soil biological conditioner, based on calcium sulfate dihydrate applied by seed treatment, during three years did not demonstrate statistically significant differences ($p < 0.05$) in BD (Figure 2a, Figure 2d). In addition, specific results for each study site can be found in Supplementary materials (Tables from S2 to S16).

However, when analyzing the biological components of soil health, there is no single tendency in SOC and ß-G indicators. For instance, there was no effect of treatment on SOC after one and two years of application at the 0–10 cm soil layer (Figure 2b). While there was a positive effect after two years of application at 10–20 cm soil layer and a negative effect of treatment on SOC after three years of application in both soil layers (Figure 2b, Figure 2e). On the other hand, the effect of treatment was positive on ß-G activity after three years of application at the 10–20 cm soil layer (Figure 2f). This particular result was driven by the performance observed at a farm located in Sarandi- RS, South region (Table S2). Furthermore, this effect was not observed at the 0–10 cm soil layer over years (Figure 2c).

There was no evidence of improvement in soil chemical indicators by the treatment after the application of the soil biological conditioner (Table 2, Figure 3). Soil pH ranged from 5.41 to 5.63 and from 4.73 to 5.21 at the 0–10 and 10–20 cm soil layers, respectively (Table 2). At the 0–10 cm soil layer, available P and exchangeable K ranged from 23.6 to 128.0 mg dm$^{-3}$ and from 90.0 to 255.2 mg dm$^{-3}$, respectively. While, available P and exchangeable K ranged from 8.4 to 66.0 mg dm$^{-3}$ and from 51.3 to 141.6 mg dm$^{-3}$ at the 10-20 cm soil layer, respectively (Table 2).

Overall, the treatment did not significantly affect the SMAF-SHI (Table 3, Figure 4). The mean SMAF-SHI values ranged from 0.66 to 0.82 for the control strips and 0.68 to 0.87 for the treatment

**Table 2.** Average of measured values of soil health indicators in three consecutive years of the soil biological conditioner application (2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) for control and treatment strips at the 0–10 and 10–20 cm soil layers

| | 0–10 cm | | 10–20 cm | |
|---|---|---|---|---|
| 2021 | Control | Treatment | Control | Treatment |
| BD (Mg m$^{-3}$) | 1.48 ± 0.15 | 1.42 ± 0.18 | 1.57 ± 0.17 | 1.54 ± 0.16 |
| SOC (%) | 2.57 ± 0.99 | 2.60 ± 1.01 | 2.03 ± 0.88 | 2.11 ± 0.78 |
| ß-G (μ PNG g$^{-1}$) | 59.8 ± 30.8 | 56.7 ± 28.1 | 25.7 ± 19.7 | 27.0 ± 17.8 |
| pH | 5.60 ± 0.55 | 5.63 ± 0.54 | 5.20 ± 0.53 | 5.18 ± 0.49 |
| P (mg dm$^{-3}$) | 61.9 ± 48.9 | 66.3 ± 57.2 | 44.6 ± 46.1 | 45.9 ± 41.3 |
| K (mg dm$^{-3}$) | 156.6 ± 93.9 | 145.7 ± 97.2 | 107.2 ± 85.0 | 104.7 ± 99.4 |
| **2022** | | | | |
| BD (Mg m$^{-3}$) | 1.38 ± 0.09 | 1.31 ± 0.09 | 1.42 ± 0.21 | 1.36 ± 0.21 |
| SOC (%) | 2.32 ± 0.41 | 2.40 ± 0.67 | 1.99 ± 0.63 | 2.38 ± 0.45 |
| ß-G (μ PNG g$^{-1}$) | 273.5 ± 227.3 | 242.8 ± 202.7 | 151.5 ± 131.6 | 137.3 ± 100.4 |
| pH | 5.44 ± 0.28 | 5.41 ± 0.42 | 5.21 ± 0.61 | 5.08 ± 0.56 |
| P (mg dm$^{-3}$) | 128.0 ± 130.0 | 102.5 ± 73.7 | 66.0 ± 79.8 | 53.9 ± 50.7 |
| K (mg dm$^{-3}$) | 232.5 ± 180.1 | 236.1 ± 221.5 | 118.5 ± 48.3 | 141.6 ± 80.2 |
| **2023** | | | | |
| BD (Mg m$^{-3}$) | 1.25 ± 0.04 | 1.20 ± 0.07 | 1.29 ± 0.13 | 1.33 ± 0.07 |
| SOC (%) | 3.06 ± 0.92 | 2.72 ± 0.85 | 2.23 ± 0.51 | 1.99 ± 0.52 |
| ß-G (μ PNG g$^{-1}$) | 260.1 ± 150.0 | 266.2 ± 138.6 | 105.0 ± 46.7 | 184.2 ± 115.6 |
| pH | 5.43 ± 0.33 | 5.72 ± 0.56 | 4.80 ± 0.23 | 4.73 ± 0.50 |
| P (mg dm$^{-3}$) | 26.9 ± 13.4 | 23.6 ± 8.7 | 8.4 ± 5.6 | 9.8 ± 6.1 |
| K (mg dm$^{-3}$) | 90.0 ± 18.5 | 255.2 ± 277.3 | 51.3 ± 14.9 | 59.6 ± 32.8 |

BD (bulk density), SOC (soil organic carbon), ß-G (ß-Glucosidase activity), pH (soil pH), P (test phosphorus), K (test potassium). Means represent all farms across the country. The plus-minus sign denotes the standard deviation of mean. Statistical analyses are reported in Figures 3 and 4.

strips respectively at the 0–10 cm soil layer, while the mean values ranged from 0.54 to 0.72 for the control strips and 0.53 to 0.78 for the treatment strips at the 10–20 cm soil layers. The chemical and biological components showed higher scores than the physical component (Table 3). This suggests that the physical component played a crucial role in contributing to the soil health gap (i.e., the difference between the current soil health index and the value of 1.0) in the strips.

A comprehensive SMAF-SHI findings for 3 out of 15 farms that performed three applications of the soil biological conditioner are shown in Figure 5. Our data showed that the effect of the soil biological conditioner on soil health was no longer detectable after three years of application. A potential benefit of the treatment for 0–10 cm soil layer was observed in the three farms, although the numerical differences were not statistically significant. In those farms, the soil was functioning from 79 to 81% and from 83 to 93% of its potential capacity at the 0–10 cm soil layer under control and treatment strips, respectively. At the 10–20 cm soil layer, the soil was functioning from 63 to 70% and from 65 to 71% of its potential capacity under control and treatment strips, respectively (Figure 5). In addition, Figure 5 shows that the contribution of the soil‘s chemical and biological components to the SMAF-SHI was higher than that of the physical component.

## Discussion

This large-scale farm-led study was the first assessment of the biological conditioner (*BLUSOIA*®) application on soil health across the 3000-km transect in Brazil‘s main soybean-producing region. Our study hypothesis was grounded on the potential effects of the soil biological conditioner in the biological activity and consequently on soil health indicators and overall soil health as well. Although the product does not contain live organisms such as microbial inoculants, the hypothesis is supported by plant–soil–microbiome interactions where the amino acid constituents of the soil conditioner (Table S1) act as a biostimulant (Moe, 2013; Soares *et al.*, 2023). However,

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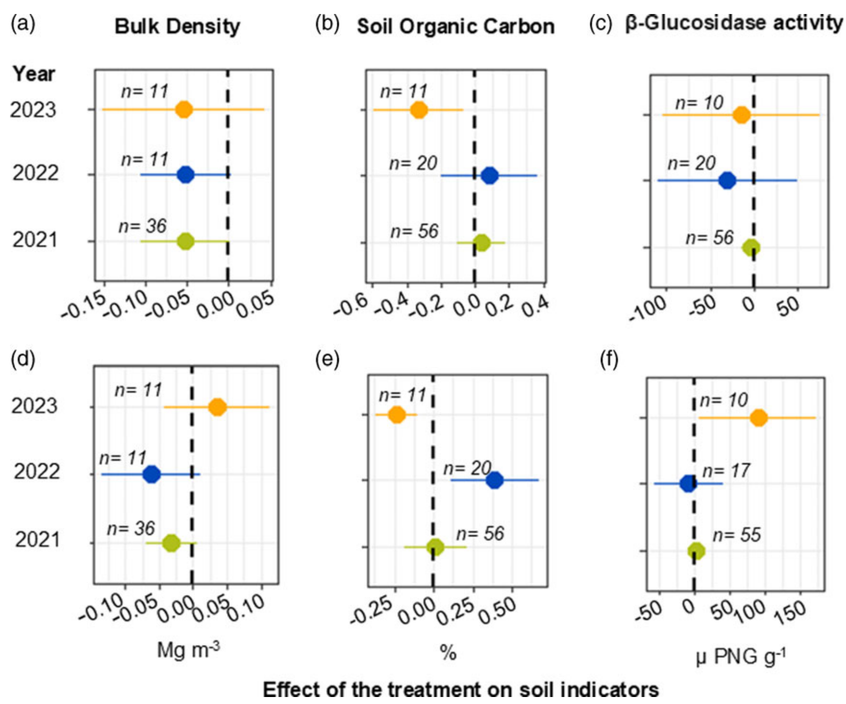


**Figure 2.** Effect of the treatment in three consecutive years of the soil biological conditioner application (2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) on soil physical (bulk density) and biological (soil organic carbon, ß-Glucosidase activity) indicators at the 0–10 (a, b, c) and 10–20 cm (d, e, f) soil layers. Solid markers are mean values of the estimated effect size and the bars are 95% confidence intervals of the treatment effect. The number of data points (*n*) for each year is indicated near the markers. Confidence intervals not overlapping zero demonstrate statistically significant differences ($p < 0.05$) resulting from the soil biological conditioner application.

our results showed that treatment with 300 g ha$^{-1}$ applied by seed dressing had no significant impact on soil health (Table 3, Figure 4). This result is supported by no clear positive changes in soil biological, chemical, and physical health indicators during three consecutive years of application (Figure 2, Figure 3). The first reason for this is the results from biological indicators. SOC and ß-G were not influenced by the soil conditioner at the 0–10 cm soil layers (Figure 2b, Figure 2c). These indicators are highly sensitive to changes induced by soil management and use (Mendes *et al.* 2019). However, their effectiveness also depends on other points such as the addition and maintenance of crop residues on soil surface and subsurface, as well as the practice of no-tillage. Similar findings were reported in a previous study by Maris et al. (2021), where the authors measured the impact of a biological conditioner based on calcium sulfate applied to maize seed dressing on soil bacteria and fungi biodiversity during a harvest event. According to the authors, no significant effect was observed on biodiversity, suggesting that the biological conditioner based on calcium sulfate did not influence soil microbial organisms.

Consequently, the treatment did not affect physical and chemical indicators. The BD was not changed by treatment at both soil layers (Figure 2a, Figure 2d). The physical indicators are dependent on other management practices such as no-tillage system and crop rotation (Moraes *et al.* 2016), cover crops and plant diversity (Hao *et al.* 2023), and machinery traffic control (Keller *et al.* 2022). On the one hand, we highlight that all farmers carried out the soybean cultivation with no tillage which contributes to the soil structure development and consequently soil bulk density reduction. On the other hand, machinery traffic, the absence of cover crops, and a lack of crop rotation can lead to an increase in soil bulk density. These factors can be associated with lower

**Table 3.** Average of measured SMAF scores for soil chemical, physical, and biological components and SMAF-SHI in three consecutive years of the soil biological conditioner application (2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) for control and treatment strips at the 0–10 and 10–20 cm soil layers

| | 0–10 cm | | 10–20 cm | |
|---|---|---|---|---|
| 2021 | Control | Treatment | Control | Treatment |
| Chemical | 0.87 ± 0.07 | 0.88 ± 0.07 | 0.79 ± 0.11 | 0.77 ± 0.13 |
| Physical | 0.43 ± 0.14 | 0.49 ± 0.20 | 0.33 ± 0.10 | 0.35 ± 0.11 |
| Biological | 0.62 ± 0.11 | 0.62 ± 0.12 | 0.53 ± 0.11 | 0.54 ± 0.12 |
| SMAF - SHI | 0.66 ± 0.08 | 0.68 ± 0.08 | 0.54 ± 0.09 | 0.53 ± 0.09 |
| **2022** | | | | |
| Chemical | 0.88 ± 0.10 | 0.88 ± 0.10 | 0.82 ± 0.09 | 0.84 ± 0.08 |
| Physical | 0.46 ± 0.13 | 0.57 ± 0.16 | 0.47 ± 0.23 | 0.55 ± 0.27 |
| Biological | 0.88 ± 0.16 | 0.88 ± 0.15 | 0.78 ± 0.18 | 0.88 ± 0.14 |
| SMAF - SHI | 0.80 ± 0.04 | 0.82 ± 0.07 | 0.72 ± 0.17 | 0.78 ± 0.15 |
| **2023** | | | | |
| Chemical | 0.87 ± 0.02 | 0.90 ± 0.06 | 0.58 ± 0.02 | 0.61 ± 0.09 |
| Physical | 0.67 ± 0.07 | 0.77 ± 0.12 | 0.61 ± 0.22 | 0.55 ± 0.13 |
| Biological | 0.90 ± 0.03 | 0.95 ± 0.02 | 0.81 ± 0.13 | 0.86 ± 0.09 |
| SMAF - SHI | 0.82 ± 0.02 | 0.87 ± 0.05 | 0.67 ± 0.03 | 0.67 ± 0.04 |

The plus-minus sign denotes the standard deviation of mean. Statistical analysis of SMAF-SHI is reported in Figure 5.

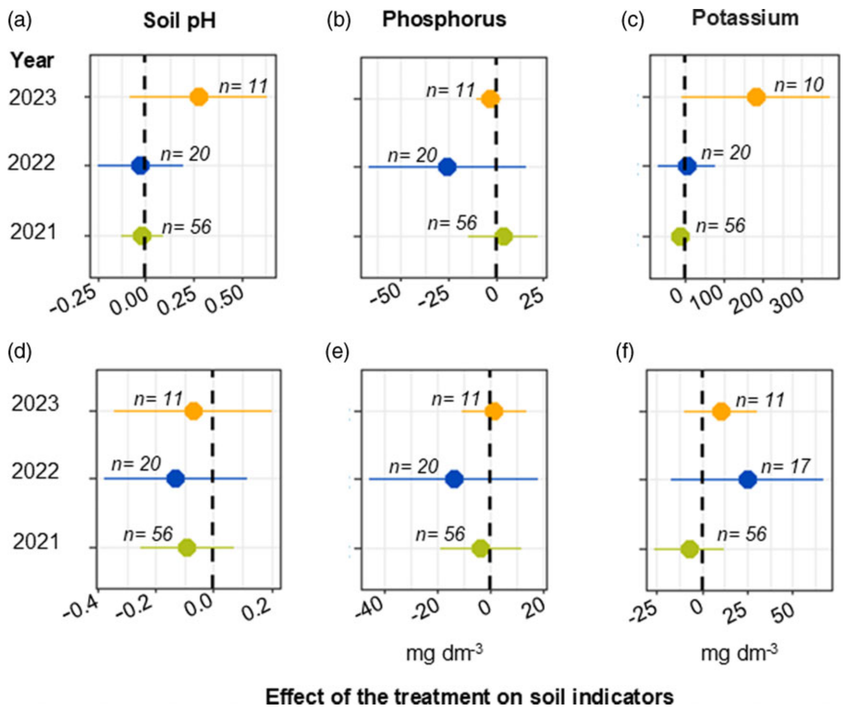


**Figure 3.** Effect of the treatment in three consecutive years of the soil biological conditioner application (2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) on soil chemical (soil pH, phosphorus, and potassium) indicators at the 0–10 (a, b, c) and 10–20 cm (d, e, f) soil layer. Solid markers are mean values of the estimated effect size and the bars are 95% confidence intervals of the treatment effect. The number of data points (*n*) for each year is indicated near the markers. Confidence intervals not overlapping zero demonstrate statistically significant differences ($p < 0.05$) resulting from the soil biological conditioner application.

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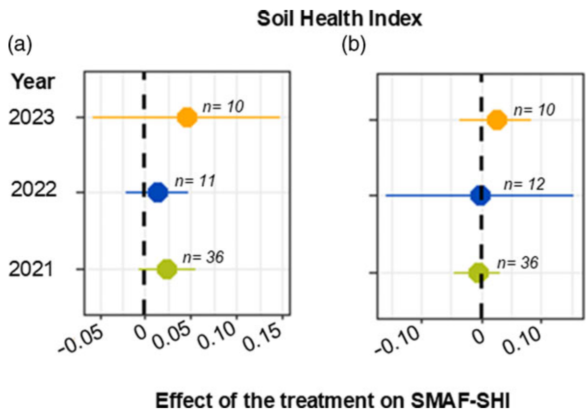


**Figure 4.** Effect of the treatment in three consecutive years of the soil biological conditioner application 2021 corresponds to one application, 2022 to two applications, and 2023 to three applications) on soil health index (SMAF-SHI) at the 0–10 (a) and 10–20 cm (b) soil layers. Solid markers are mean values of the estimated effect size and the bars are 95% confidence intervals of the treatment effect. The number of data points (*n*) for each year is indicated near the markers. Confidence intervals not overlapping zero demonstrate statistically significant differences ($p < 0.05$) resulting from the soil biological conditioner application.

SMAF scores of the soil's physical components compared to those of the soil's chemical and biological components (Table 3, Figure 5). The use of a soil biological conditioner based on calcium sulfate could improve soil physical health by enhancing root development, especially fine and medium roots (Maris *et al.* 2021), as well as promoting the formation of soil aggregates, bio-pores (Vogel *et al.* 2021), and stimulating root exudation. Results from specific locations (e.g., Figure 5a, Figure 5c) suggest potential improvements in soil physical health at the 0–10 cm soil layer with the application of the soil biological conditioner (Table S2, Table S3). Therefore, continuous monitoring over the next few seasons is recommended to find changes in soil physical health.

The soil biological conditioner could affect the soil chemical indicators by increasing soil biological activity which acts on nutrient transformation, nutrient reallocation, and nutrient assimilation (Creamer *et al.* 2022). However, our findings showed that the application of the soil biological conditioner did not affect soil pH, available P, and exchangeable K (Table 2, Figure 3). These indicators respond to various agricultural practices such as lime application (e.g. soil pH) and fertilizer application (e.g., available P and exchangeable K). In both the control and treatment strips, soil pH was near 5.5. A soil pH near this value can enhance the efficiency of fertilization and the availability of soil nutrients (Winck *et al.* 2023). Therefore, the chemical indicator values found in the strips did not change as a result of the soil biological conditioner application.

Currently, the fast growth in the adoption of biological inputs in Brazil presents several challenges (Soares *et al.* 2023). The first one is the application and monitoring of biological inputs to ensure their effectiveness in agriculture. The second one is fostering collaborative efforts among universities, research institutions, and specialized companies in agricultural biotechnology. The third one is ensuring the efficient and safe use of biological technologies for large-scale agricultural production. Our study was pioneering to face these challenges and demonstrated promising results at a large scale. Additionally, since there was no significant difference between the strips, we recommend further studies focusing on the rhizosphere environment and testing different application rates. The rhizosphere, the area around plant roots, is distinguished by faster interactions among plants, roots, and organisms compared to the bulk soil (Hartmann *et al.*, 2008; Poupin, 2024). It is also recognized as one of the most important microbial hotspots in the soil (Kuzyakov & Blagodatskaya 2015) and is characterized by more intense chemical, physical, and biological interactions than the surrounding soil(Vogel *et al.* 2021). Therefore, the plant-soil-microbiome

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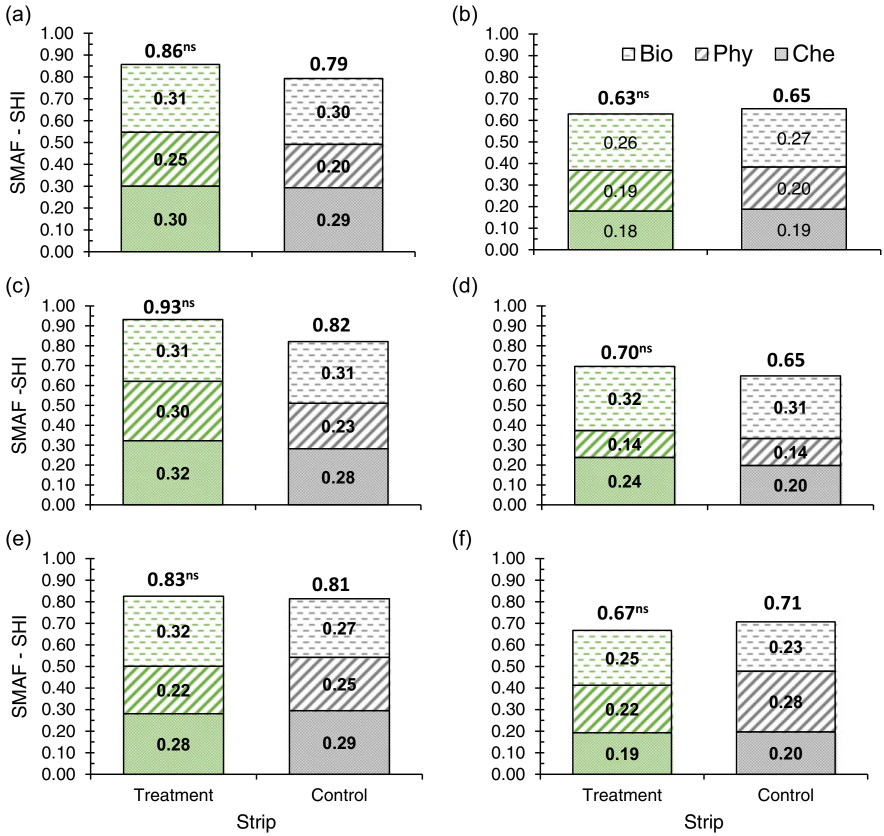


**Figure 5.** SMAF – Soil health index (SHI) and weighted contribution of the biological (Bio), physical (Phy), and chemical (Che) components for the overall soil health after three consecutive years of the soil biological conditioner application for control and treatment strips at the 0–10 (a, c, e) and 10–20 cm (b, d, f) soil layers. The farms that applied the soil biological conditioner in three consecutive years were located in Quatro Irmãos-RS, South region (a, b), Sarandi-RS, South region (c, d), and Sorriso-MT, Midwest region (e, f). $^{ns}$ The mean difference between strips within each site and layer is not significant according to Tukey's test ($p < 0.05$).

interactions in the rhizosphere environment could respond to the soil conditioner applied by seed dressing. On the other hand, our results showed that soil health indicators did not respond to the application rate of 300 g ha$^{-1}$. This suggests that future studies should explore other application rates, evaluate soil health responses in the rhizosphere environment over long-term years, and include molecular biology methods to assess the microbiome in areas treated with soil biological conditioners.

## Conclusion

The results of farm-led experiments across 15 sites and three years revealed that the soil biological conditioner, based on calcium sulfate dihydrate and applied by seed treatment, caused site- and year-specific alterations on soil chemical, physical, and biological indicators, as well as overall soil health. On average, the effects were subtle and statistically undetectable for most of the metrics. However, we highlight potential changes in soil organic carbon, extracellular β-glucosidase enzyme activity, and soil bulk density indicators as influenced by the soil biological conditioner. To further understand the effects of biological conditioners on soil, we propose continuing soil

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health monitoring over time, with a particular focus on the rhizosphere, and include molecular biology methods to assess the abundance, diversity, and functionality of the soil microbiome in areas treated with biological conditioners.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0014479725000080

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