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Soil microbiological attributes and sugarcane productivity following implementation of three sugarcane reformation systems

Atributos microbiológicos do solo e produtividade da cana-de-açúcar após a implementação de três sistemas de reforma

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Highlights

Crotalaria spectabilis used as cover crop before sugarcane, enhances soil microbiology.
Soil with *C. spectabilis* showed increased microbial biomass carbon and glomalin levels.
Reformed systems with cover crops increased sugarcane productivity.

Abstract

The increasing demand for biofuels has driven the Brazilian sugarcane industry to expand into degraded pasture areas with low organic matter content and fertility. Traditionally, sugarcane is cultivated in sandy soils, and after five or more harvest cycles, field reform involves conventional tillage, followed by sugarcane planting in the exposed soil. However, the introduction of cover crops during this reform period has shown soil fertility benefits, although research on soil microbiology impacts is limited. This study aimed to evaluate soil microbiological attributes and sugarcane productivity following the implementation of three different reform systems in a sandy Ultisol. The three systems assessed were conventional planting in exposed soil and field reform using either soybean or *Crotalaria spectabilis*. Ten samples were randomly collected from a 10-hectare plot at a depth of 0.00–0.10 m, near the planting furrow, for each management system. The samples were analyzed for organic carbon, microbial biomass carbon, total glomalin, easily extractable glomalin, and sugarcane productivity. Data were subjected to an analysis of variance and means were compared using Tukey's test. The findings indicate that cultivating *C. spectabilis* before planting sugarcane enhances soil health and mitigates the impacts

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of agricultural practices. This improvement is attributed to higher levels of microbial biomass carbon, easily extractable glomalin, and total glomalin, which contribute to increased sugarcane productivity.

Key words: Sandy Ultisol. *Saccharum officinarum* L. Microbial biomass carbon. Glomalin. Stalk productivity.

Resumo

A crescente demanda por biocombustíveis tem levado a indústria brasileira de cana-de-açúcar a expandir suas atividades para áreas de pastagens degradadas, caracterizadas por baixa matéria orgânica e fertilidade. Tradicionalmente, a cana-de-açúcar é cultivada em solos arenosos e, após cinco ou mais ciclos de colheita, realiza-se a reforma do campo, que inclui o preparo convencional do solo seguido pelo plantio da cana em solo exposto. Contudo, a introdução de uma cultura de cobertura durante esse período de reforma tem mostrado benefícios para a fertilidade do solo, embora haja poucos relatos sobre os impactos dessa prática na microbiologia do solo. Este estudo tem como objetivo avaliar os atributos microbiológicos do solo e a produtividade da cana-de-açúcar após a implementação de três sistemas de reforma em um Argissolo Vermelho-Amarelo. Os sistemas avaliados foram: o plantio convencional em solo exposto, e a reforma do campo com cultivo de soja ou *Crotalaria spectabilis.* Foram coletadas dez amostras aleatórias de uma parcela de 10 hectares, na camada de 0,00 - 0,10 m, para cada sistema de manejo, próximo ao sulco de plantio. As amostras foram analisadas para determinar o carbono orgânico, o carbono da biomassa microbiana, a glomalina total e a glomalina facilmente extraível, além da produtividade da cana-de-açúcar. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey ($p \leq 0{,}05$). Os resultados indicam que o cultivo de *C. spectabilis* antes do plantio da cana-de-açúcar melhora a saúde do solo e mitiga os impactos das práticas agrícolas. Esse benefício é atribuído aos níveis elevados de carbono da biomassa microbiana, glomalina facilmente extraível e glomalina total, que contribuem para uma maior produtividade.

Palavras-chave: Argissolo arenoso. *Saccharum officinarum* L. Carbono da biomassa microbiana. Glomalina. Produtividade do colmo.

Introduction

Understanding the differences in agricultural practices that promote and enhance soil quality is crucial for sustainable production. Microbiological attributes are more sensitive indicators of management practices than are physical and chemical characteristics (Garcia et al., 2020; Cagnini et al., 2019). This sensitivity arises from the reliance of microbiological indicators on factors such as gas, water, and nutrient flow, as well as their links to soil fertility, structure, and aggregation. Changes in these factors can lead to variations in biological attributes, affecting crop development, and consequently, crop productivity. Thus, researchers have suggested that the microbiological indicators commonly used to monitor soil environmental changes are valuable tools for evaluating soil quality and guiding agricultural management practices to improve production.

Indicators such as organic carbon (OC), microbial biomass carbon and nitrogen, soil basal respiration, microbial quotient, and metabolic quotient are essential for tracking changes in soil management over time. The use of glomalin an important microbiological characteristic that varies based on management practices (Garcia et al., 2020) provides insights into the effects of management on soil stability, carbon sequestration, biological activity, and crop productivity. Conservation management systems such as crop rotation and the use of green manure promote more stable soil aggregates and higher glomalin levels.

In response to the growing demand for biofuels, the Brazilian sugarcane industry has expanded to degraded pastures with low organic matter content and fertility (Oliveira et al., 2019). Traditionally, sugarcane fields have been reformed using conventional tillage, followed by sugarcane planting in exposed soil after five or more harvest cycles (Moraes et al., 2022). However, these soils are highly susceptible to degradation and erosion (Garcia et al., 2020). Alternative management techniques such as the incorporation of soybeans or cover crops before planting sugarcane have been proposed. The use of cover crops in conventional sugarcane reform systems can improve soil moisture, organic matter content, and microbial activity, all of which may enhance productivity (Marshall & Lynch, 2020). However, selecting the most beneficial crop for the reform period remains challenging, as there is limited information on the effects of different crops on soil quality indicators, particularly microbiological indicators. Thus, this study aimed to evaluate soil microbiological characteristics and sugarcane productivity following the implementation of three different reform systems in a sandy Ultisol.

Materials and Methods

The study was conducted under a sandy Ultisol (795, 105, and 100 g kg^{-1} of sand, silt, and clay, respectively) in Brazil at latitude 21° 13' 40"S, longitude 50° 52' 06"W, and altitude 449 m. The biome of the region is the "cerrado" type, and the climate, according to the Köppen classification, is Aw (tropical humid with dry winter). It has maximum, average, and minimum annual temperatures of 31, 24, and 18 °C, respectively, annual precipitation volumes of 1200–1500 mm, and rainfall <60 mm in the coldest dry month (Moraes et al., 2022).

A sugarcane production area previously used as a pasture was selected. The land had been used for sugarcane cultivation since 2000, with burned sugarcane used in the first decade and raw sugarcane used in subsequent years. The crops were planted at a spacing of 1.50 m between the grooves for five harvest cycles. Subsequently, the area was reformed using conventional preparation methods, including chemical desiccation of the cane ratoon, heavy grading, intermediate grading, subsoiling, and grading leveling. Revolving soil operations were applied to correctives (limestone: 2.3 Mg ha^{-1} and gypsum: 1.5 Mg ha^{-1}) and by-product application (vinasse: 1.5 m^3 ha^{-1} and filter cake: 3.5 m^3 ha^{-1}).

The area was then divided into three subareas of 10 ha, the management of which was as follows: area 1, sugarcane planted in exposed soil (conventional tillage); area 2, sugarcane planted in the ratoons after soybean cultivation in the no-tillage system for 5 months (conventional tillage + soybean); and area 3, sugarcane planted in the green mass resulting from the planting of *Crotalaria spectabilis* 5 months after sowing and incorporation with a leveling harrow (conventional tillage + *C. spectabilis).*

To analyze the effects of these systems on soil microbiological attributes, soil samples were collected during the third cropping cycle, 12 months after implementation of the new practices. We randomly collected ten samples from each management area, covering 10 ha, with one sample collected from each hectare near the planting furrow, at the 0.00–0.10 m layer. These samples were analyzed for OC, microbial biomass carbon (MBC), total glomalin (TG), easily extractable glomalin (GEE), and sugarcane productivity.

The OC and MBC were determined using the Walkley–Black method (Fontana & Campos, 2017) and the modified fumigation-extraction method (Babujia et al., 2010), respectively, using non-fumigated and fumigated samples. The fumigated samples were placed in a vacuum box with 50 mL of chloroform at each apex for 16 h. The non-fumigated samples were treated similarly but with 50 mL of distilled water. After incubation, the samples were suspended in 50 mL of extractor solution (K_2SO_4 0.5 M). MBC concentrations in the extracts were determined by oxidation with Mn^{3+} and estimated colorimetrically at a wavelength of 495 nm (Bartlett & Ross, 1988). The MBC of the extracts was calculated by determining the difference between the fumigated and non-fumigated samples using a correction factor of 0.41, as recommended for tropical soils.

To extract glomalin GEE and TG, different conditions and quantification methods were used (Rillig, 2004). GEE was obtained from autoclave extraction using 1 g of soil and 8 mL of sodium citrate solution 20 mM (pH 7.4) at 121 °C for 30 min. TG was obtained using 1 g of soil and 8 mL of sodium citrate 50 mM, with pH 8.0, at 121 °C for 60 min. More than one autoclaving cycle (3–10 cycles, depending on the sample) was required to extract the TG fraction until the sample reached a light-yellow color. After autoclaving, centrifugation was performed at 5,000 x g for 20 min, and the supernatant was removed for protein quantification. The Bradford method (Bradford, 1976) was used to quantify glomalin, using bovine serum albumin as the standard. The glomalin concentrations for both fractions were corrected for mg g-1 of soil, considering the total volume of the supernatant and the dry mass of the soil.

The data from each evaluated layer underwent normality and homoscedasticity tests using the Shapiro–Wilk and Bartlett’s tests, respectively. Next, the analysis of variance was conducted using an F-test ($p \leq 0.05$) in a completely randomized design mode, and the means were compared using Tukey’s test ($p \leq 0.05$). Pearson's linear correlation coefficient was used to perform correlation tests between soil biological properties. All statistical analyses were conducted using R software (R Core Team [R], 2021).

Results and Discussion

The results indicate that the microbiological attributes of the soil were influenced by the type of sugarcane reform system implemented (Table 1A). Specifically, the reform system that incorporated *C. spectabilis* as a cover crop showed significantly higher levels of OC, MBC, GEE, and TG than the other reform systems. In contrast, the conventional tillage system with soybean resulted in lower OC, MBC, GEE, and TG levels. While there were no significant differences in OC and MBC between the conventional tillage system with soybean and the conventional tillage reform system, notable differences were observed in TG and GEE. These findings were consistent with those reported by C. F. Silva et al. (2012) and Oliveira et al. (2019).

Table 1

Organic carbon (OC), microbial biomass carbon (MBC), total glomalin (TG), easily extractable glomalin (GEE); levels in the 0–10 cm sandy Ultisol layer, under different management systems: conventional tillage (CT), conventional tillage + soybean (CT+S), and conventional tillage + C. spectabilis (CT+C) (A). Pearson correlation (r2) for biological indicators as a function of management under a conventional sugarcane reform system (n = 30). (B)

(A)				
	OC (g dm^{-3})	MBC (mg kg^{-1})	TG (mg kg^{-1})	GEE (mg kg^{-1})
CT	3.80B*	340.8B	2.44B	1.10B
CT+S	3.70B	330.2B	1.67C	0.79C
CT+C	8.60A	408.8A	2.66A	1.35A
(B)				
	MBC	GEE	TG	PROD
MBC	1	0,86*	0,69*	0,75*
GEE		1	0,87*	0,14
TG			1	0,38
PROD				1

*Means compared by the Tukey test (p-value < 0.05). * P value ≤ 0.01.

Pearson's correlation analysis revealed positive correlations among the biological indicators: MBC was positively correlated with both easily extractable glomalin GEE, TG, and stalk productivity, while GEE exhibited a positive correlation with TG only (Table 1B).

The analysis of stalk production per hectare (Figure 1) revealed significant differences among the three reform systems. The conventional tillage + Crotalaria cover reform system achieved the highest production with 148.77 tons of stalks per hectare. This was followed by the conventional tillage + soybean and conventional tillage reform systems which produced 122.03 and 87.90 tons of stalks per hectare, respectively. These findings are consistent with those of Moraes et al. (2023).

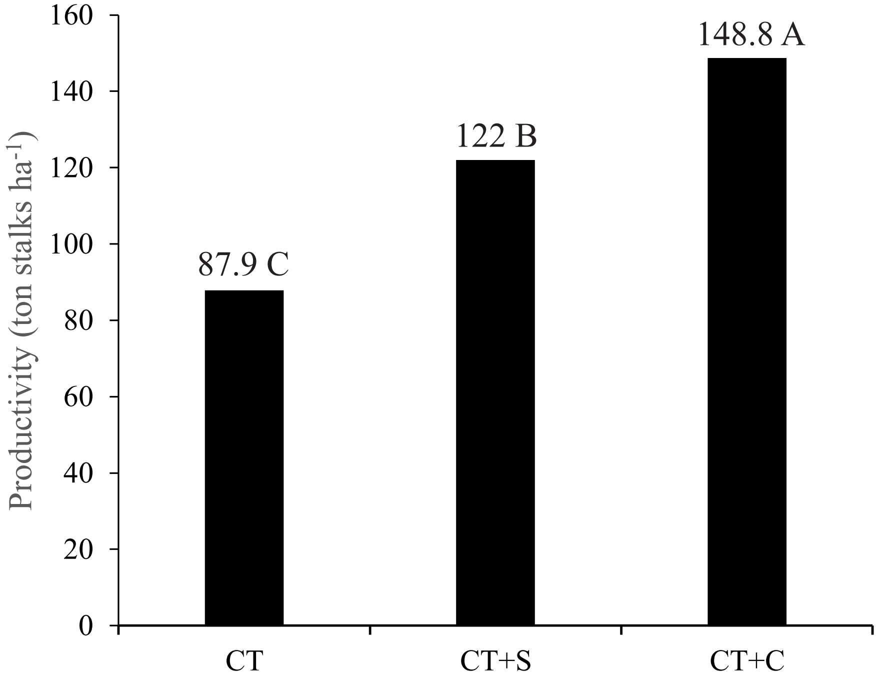

Figure 1. Productivity of sugarcane stalks ha^{-1} under different management systems: conventional tillage (CT), conventional tillage + soybean (CT+S), and conventional tillage + *C. spectabilis* (CT+C) in a sandy Ultisol.

The higher levels of OC, MBC, TG, and GEE observed in the conventional tillage + *Crotalaria* cover cropping system suggest that using *C. spectabilis* as a cover crop before planting sugarcane provides significant benefits to soil carbon content and microbiological attributes. A tropical legume, C. spectabilis contributes substantial green mass (~7 Mg per hectare (Barbosa et al., 2020)), improving soil conditions by moderating temperature and humidity fluctuations, enhancing biological activity, and increasing carbon content despite its short residence time in the soil (Moraes et al., 2023). This increased OC promotes better soil quality, aggregation, and the formation of complex organic acids (Canellas et al., 2015), which help attenuate rapid organic matter decomposition.

Although the potential of *C. spectabilis* to boost soil organic matter is recognized, its effectiveness depends on several factors, including residue quality, C/N ratio, soil conditions, and the presence of recalcitrant substances such as lignin and phenolic compounds (Canellas et al., 2015). The C/N ratio of C. spectabilis is typically ~13:16 (Perin et al., 2010), facilitating rapid decomposition and nitrogen release, which benefits subsequent crops. This might explain the rapid growth of sugarcane in this system. Additionally, *C. spectabilis* effectively reduces nematodes and fixes atmospheric nitrogen, thereby contributing to increased productivity (Moraes et al., 2023).

The other management systems had lower OC levels, likely due to greater organic matter oxidation. In conventional tillage, sugarcane is planted in bare soil after disturbance (Moraes et al., 2023). The conventional tillage + soybean system with its lower green mass production resulted in faster decomposition and mineralization of soil OC. However, this system still achieved a higher productivity (122.03 tons of stalks per hectare) than conventional tillage alone likely because of biological nitrogen fixation by soybeans underscoring the value of incorporating legumes during sugarcane reform. The conventional system had an average stalk productivity similar to the national average (85.6 tons ha^{-1}) according to Companhia Nacional de Abastecimento [CONAB] (2024), and the other systems had improved productivity compared to the national average and the conventional system.

Regarding microbiological attributes, the highest levels of MBC, GEE, and TG were observed in the conventional tillage + *Crotalaria* system, indicating that this is the most effective management approach for enhancing these attributes. Microbial carbon content reflects soil quality and responds sensitively to management changes (Babujia et al., 2010; A. P. Silva et al., 2014; Garcia et al., 2020). Glomalin, a protein associated with soil aggregate stability and containing carbon and nitrogen, varies with microbial activity and management practices (Zhao et al., 2022; Sousa et al., 2012). Changes in microbial communities occur more rapidly than changes in physical and chemical attributes (Hurisso et al., 2013), highlighting the importance of monitoring these indicators to understand their impacts on soil quality.

This study suggests that the conventional tillage + *Crotalaria* system fosters favorable conditions for microbial growth and activity. The root system and exudates of *C. spectabilis* enhance soil particle aggregation and microbial activity (Cagnini et al., 2019). Higher sugarcane productivity in this system indicates improved soil health and fertility, likely due to enhanced microbial activity, which supports aggregate formation, plant nutrition, and root protection.

The positive correlation between MBC and glomalin highlights its impact on soil aggregation in sandy soils, consistent with previous findings (Zhao et al., 2022; Sousa et al., 2012). The higher levels of these attributes in the conventional tillage + *Crotalaria* system suggest that *C. spectabilis* mitigated the adverse effects of conventional tillage, leading to better soil conditions and increased sugarcane productivity of 148.77 tons of stalks per hectare. Thus, incorporating *C. spectabilis* into the sugarcane reform system improved soil OC levels, microbiological attributes, and overall productivity.

Conclusion

After evaluating three different methods of sugarcane cultivation in sandy Ultisols, it was found that the most effective approach was the use of conventional tillage combined with a *C. spectabilis* cover crop prior to planting sugarcane. This method produced the highest levels of OC, microbial biomass carbon, total glomalin, and easily extractable glomalin in the soil. Moreover, this treatment resulted in the highest stalk production, reaching 148.77 tons per hectare. These results highlight the benefits of incorporating green cover crops such as *C. spectabilis* into the sugarcane cultivation process. The presence of cover crops enhances soil quality by promoting microbial activity, improving soil structure, and ultimately leading to increased productivity per hectare.

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Author contributions

LAAM, APS, and JTF designed experiments. GMO and LAAM prepared the samples and GMO performed the experiments. APS and GSM analyzed the data and wrote the manuscript. GSM and JTF reviewed and checked all details. All the authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the study design; collection, analyses, or interpretation of data; writing of the manuscript; or decision to publish the results.

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