

Research Article

Biostimulants For Climate-Smart Farming: Improving Carbon Retention And Yield In Maize Systems.

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Agricultural intensification has contributed to rising greenhouse gas emissions, particularly carbon dioxide (CO₂), highlighting the need for management strategies that enhance carbon retention while maintaining crop productivity. Soil biostimulants that stimulate microbial activity and plant growth may help improve carbon-use efficiency in agricultural systems. This study evaluated the effects of a microbial biostimulant formulation on soil biological activity, carbon dynamics, and maize productivity. The research was conducted in three stages: (i) laboratory incubation to assess microbial activity and soil respiration under different biostimulant dosages; (ii) field evaluation of CO₂ emissions in maize cultivated with and without the biostimulant; and (iii) assessment of plant development and carbon retention in biomass during the crop cycle. Biostimulant application increased microbial activity and plant biomass production, resulting in greater carbon retention in plant tissues. Although soil respiration increased in treated soils, higher biomass production partially offset carbon losses by increasing carbon incorporation into plant and microbial biomass. When emissions were analyzed relative to grain yield, the treated system showed improved carbon-use efficiency, with lower CO₂ emissions per unit of grain produced compared with the control. These results suggest that microbial biostimulant applications may contribute to climate-smart agricultural practices by enhancing soil biological functioning, improving crop productivity, and increasing carbon retention in agroecosystems. However, long-term and multi-site studies are needed to better quantify net carbon balances and to assess the broader climate mitigation potential of such approaches.

Keywords: Low-carbon agriculture; Microbial biostimulant; Soil biological quality; Agricultural sustainability; Climate change mitigation Low-carbon agriculture; Microbial biostimulant; Soil biological quality; Agricultural sustainability; Climate change mitigation.

INTRODUCTION

The contribution of agriculture to the intensification of the anthropogenic greenhouse effect can be reduced through the adoption of soil management systems capable of mitigating greenhouse gas emissions. In this context, mitigation refers to anthropogenic interventions aimed at reducing emissions or enhancing greenhouse gas sinks. Interest in this topic has grown considerably within the global scientific community. Given the strong dependence of agricultural development and crop productivity on climatic conditions, agriculture is among the sectors most vulnerable to the impacts of climate change, with potentially severe consequences for global food security [1, 2]. Although projections of rising temperatures and their impacts on natural ecosystems and agroecosystems remain uncertain, anticipated consequences include increased global temperatures, sea-level rise, more frequent periods

of water surplus and deficit, and shifts in regions suitable for agricultural production. Considering the central role of carbon dioxide (CO₂) in the intensification of the greenhouse effect, identifying strategies that reduce agricultural carbon emissions while enhancing CO₂ fixation in crops is essential. Soil organic carbon stocks are determined by the balance between carbon inputs derived from photosynthesis (inflow) and carbon losses through the oxidation of organic matter to CO₂ by heterotrophic microorganisms (efflux) [3]. Therefore, changes in soil organic carbon stocks can serve as indicators of the effects of soil management practices, products, and technologies on the net carbon balance of the soil-atmosphere system.

Estimating net carbon fluxes—whether efflux or influx—based on changes in soil organic carbon stocks is an effective approach for evaluating the long-term impacts of management systems on climate change mitigation. This

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method is recommended by the Intergovernmental Panel on Climate Change (IPCC) for greenhouse gas inventories in agricultural systems [4]. It is also considered a suitable framework for assessing soil carbon sequestration under regenerative agriculture systems, which may contribute to certified emission reduction initiatives and carbon market mechanisms.

Within this environmental context, regenerative agriculture has emerged as a promising model for supporting food production while reducing environmental impacts [5]. This perspective has contributed to the development of so-called low-carbon agriculture, which aims to reduce greenhouse gas emissions associated with agricultural activities. Such approaches have gained increasing attention within global environmental policy discussions following international agreements under the United Nations Framework Convention on Climate Change, through which countries have committed to reducing the environmental impacts of production systems. Several studies have reported that soil and plant biostimulants can enhance soil biological quality and crop productivity, potentially increasing carbon incorporation into plant tissues through greater biomass production [6–11]. Enhanced root growth and shoot development may contribute to greater carbon inputs into soil and plant systems, thereby influencing carbon cycling in agroecosystems. However, the extent to which such inputs affect net carbon emissions and carbon-use efficiency under field conditions remains insufficiently documented, particularly in commercial production systems. Studies that quantify carbon dynamics through measurements of soil CO₂ emissions and plant biomass production under different management strategies are therefore essential for evaluating the environmental performance of agricultural systems. In this context, the present study aimed to assess microbial activity and CO₂ emissions under laboratory and field conditions in a maize cultivation system managed with and without the application of microbial biostimulants.

MATERIAL AND METHODS

The study was conducted in three stages: (i) evaluation of microbial activity stimulation using a soil-applied biostimulant (SoilPlus®); (ii) assessment of field CO₂ emissions in maize cultivated with soil- and foliar-applied biostimulants (Soilplus® and FieldStim®); and (iii) evaluation of maize growth and carbon sequestration in plant tissues during the crop cycle, with and without biostimulant application. The products SoilPlus® and FieldStim® were supplied by PENERGETIC International (Switzerland).

In the first stage, soil was collected from a grain production area, sieved through a 2-mm mesh, and homogenized for treatment preparation. Three treatments were evaluated: (1) control (without biostimulant application); and (2)

soil application of the biostimulant at rates equivalent to 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 g ha⁻¹, combined with a foliar-applied biostimulant at 500 g ha⁻¹. After incorporation of the products, soil samples (100 g at 80% of field capacity) were placed in airtight glass bottles, with gas exchange allowed every 48 hours. Soil moisture was maintained by adding water at seven-day intervals. After 45 days of incubation, basal respiration, β-glucosidase enzyme activity, and microbial cell counts were evaluated.

Based on the results of the initial incubation tests, a selected dosage was used for extended evaluation of biological activity and basal respiration over a 150-day incubation period. Basal soil respiration was determined following the methodology described by Francioni et al. [12]. Gas emissions were quantified using an adaptation of the method proposed by Courtois et al. [13], employing a LI-COR Biosciences-based sensor coupled to sealed glass incubation chambers. Results were expressed as kilograms of carbon in the form of CO₂ per hectare per day. β-glucosidase activity was measured according to Tabatabai [14], using colorimetric determination of p-nitrophenol released after incubation with p-nitrophenyl-β-D-glucopyranoside. Microbial cell counts were performed using the colony-forming unit (CFU) method described by Bakken and Olsen [15].

Following the laboratory phase, the second and third stages were conducted in a maize field located at the experimental area of Biomonte Research and Development in Santa Maria, Rio Grande do Sul State, Brazil. The experiment followed a randomized block design with split plots. The maize hybrid used was DKB235PRO3, sown at a population density of 75,000 plants ha⁻¹.

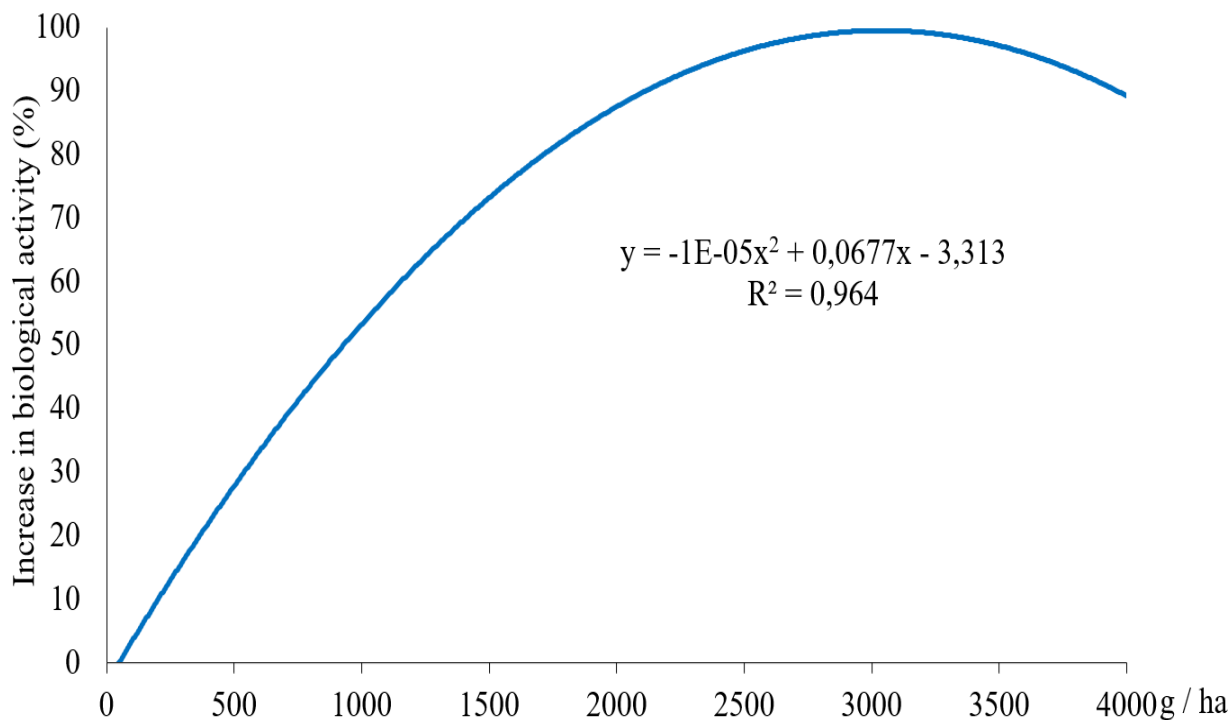
Fertilization was based on soil chemical analysis and followed regional recommendations for maize cultivation. At sowing, 185 kg ha⁻¹ of a 15-20-20 (N-P-K) formulation was applied, supplying phosphorus (P₂O₅) and potassium (K₂O). Supplemental nitrogen fertilization (urea) was applied at a rate of 20 kg N ha⁻¹ at the BBCH 15 growth stage.

Experimental units were divided into two treatments: (1) control (without biostimulant application); and (2) biostimulant treatment consisting of soil application at 2750 g ha⁻¹ applied 15 days before sowing, followed by a single foliar application at 500 g ha⁻¹ at the BBCH 14 growth stage. The following parameters were evaluated: root and shoot dry mass (45 and 120 days after emergence) and basal soil respiration (45 and 120 days after emergence). In situ soil respiration was determined using gas capture chambers adapted from the laboratory incubation method. Data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at the 5% significance level with the SISVAR statistical software [16].

RESULTS AND DISCUSSION

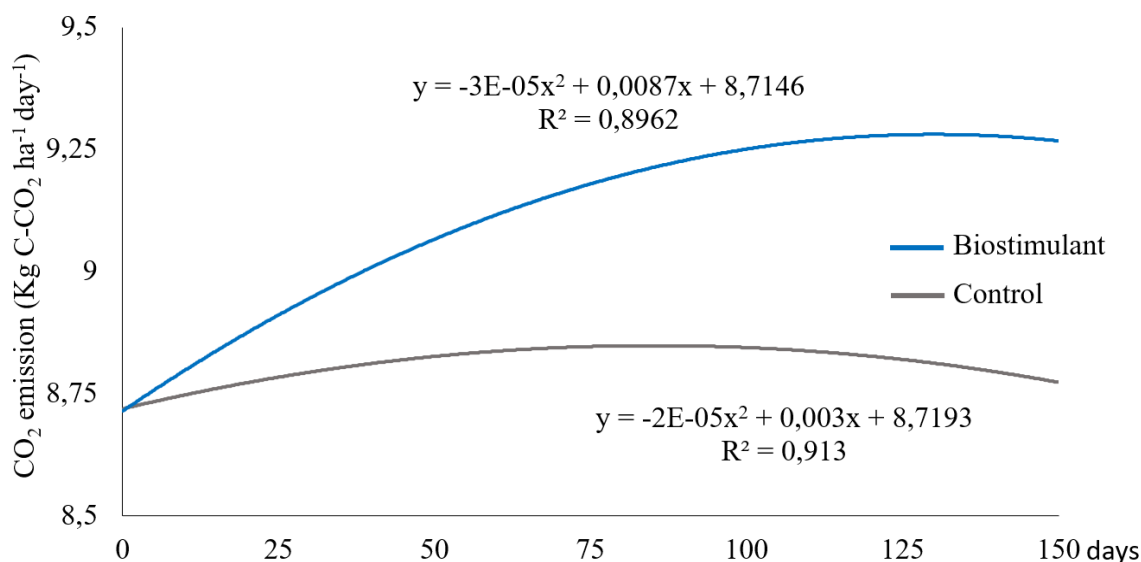
The stimulation of microbial activity induced by the SoilPlus® biostimulant followed a dose-response relationship. Based on the mathematical models fitted to the response curves of the different treatments, maximum efficiency was observed at an application rate of 2750 g ha⁻¹ (Figure 1).

Figure 1. Biological activity assessed by the activity of the β -glucosidase enzyme in soil incubated in the different dosage in the biostimulant. Comparative results to control treatment.



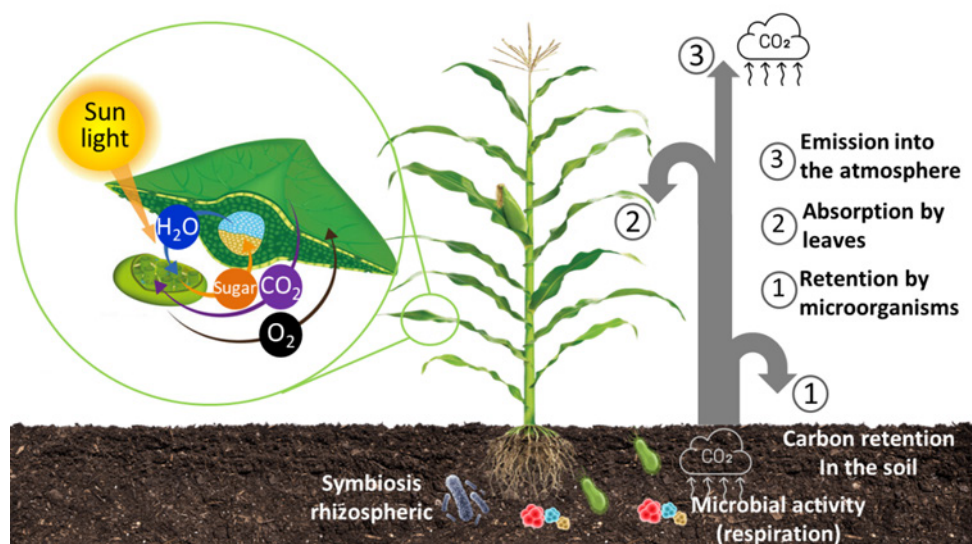
Based on previous knowledge and earlier studies, this dose-response relationship appears to be related to the initial biological quality of the soil. Soils with higher baseline biological activity may require lower application rates to stimulate microbial processes, whereas soils with lower biological quality may require higher dosages to achieve comparable responses. However, the greater microbial activity observed with the powdered formulation did not correspond to higher CO₂ emissions under controlled conditions (Figure 2).

Figure 2. Carbon emission in the C-CO₂ form in different treatments in incubated soil tests.



Microbial activity in soil is closely linked to basal soil respiration (CO_2 emissions) [3,17–19]. Several authors emphasize the importance of considering both carbon efflux (emission) and influx (retention) processes within soil–plant systems. Stimulation of the soil microbiota may lead to either increased carbon emission or enhanced carbon retention, as part of the carbon that would otherwise be released into the atmosphere can be incorporated into newly formed microbial cells through microbial growth or into plant tissues via photosynthesis (**Figure 3**).

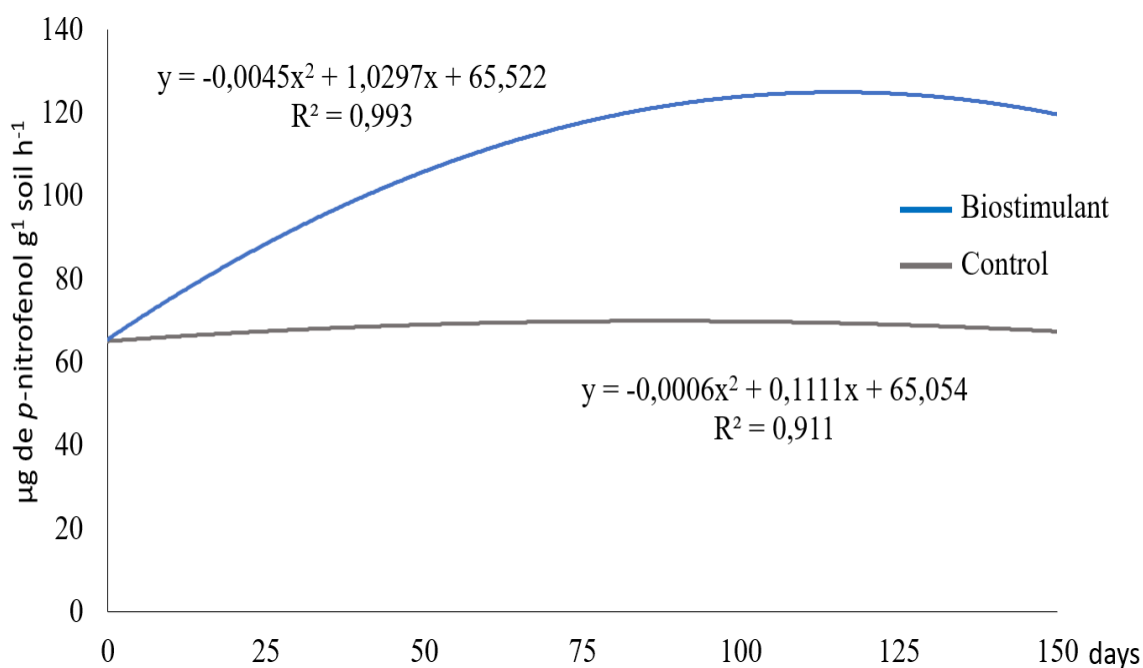
Figure 3. Efflux (emission) and influx (retention) cycles in the soil-plant-atmosphere system. Source: Authors.



In other words, microbial stimulation is often associated with an increase in microbial population size. As microbial biomass expands, a portion of the carbon that might have been emitted as CO_2 is instead assimilated into microbial biomass, contributing to carbon retention in the system. Nevertheless, some CO_2 release still occurs as a result of microbial metabolic processes accompanying the formation of new cells.

In the evaluations conducted over 150 days, a response pattern similar to that observed in the initial *in vitro* tests was confirmed. The SoilPlus® treatment applied at a rate of 1500 g ha^{-1} resulted in the highest microbial activity, as indicated by β -glucosidase enzyme activity throughout the evaluation period. This treatment was significantly more effective in stimulating microbial activity than the control, as demonstrated by the consistently higher β -glucosidase activity in treated samples (**Figure 4**).

Figure 4. Microbial activity assessed by β -glucosidase enzyme in different treatments in incubated soil tests.



Mathematical models fitted to the response curves indicate that peak microbial activity occurred between 90 and 120 days after application. This finding is relevant for optimizing application timing, as it allows synchronization of peak soil biological activity with periods of maximum physiological demand in the crop.

Based on the results obtained from maize development under field conditions, it was possible to estimate the carbon balance among the different treatments. Vegetation, whether native or cultivated, plays a fundamental role in regulating atmospheric carbon dioxide concentrations through processes such as photosynthesis, respiration, and decomposition, all of which influence the consumption and release of CO₂—currently the most significant greenhouse gas in the context of climate change. Through photosynthesis, plants fix atmospheric CO₂ by converting it into carbohydrates that are subsequently incorporated into structural components of plant tissues, a process referred to as carbon sequestration. In the present study, biostimulant application increased root biomass (**Figure 5**) as well as aboveground biomass (stems, leaves, and ears) compared with the control at both 45 and 120 days after emergence (**Figure 6**).

Figure 5. Weight of maize roots and increase in different treatments at 45 and 120 days after emergence. Considering 75,000 plants per hectare.

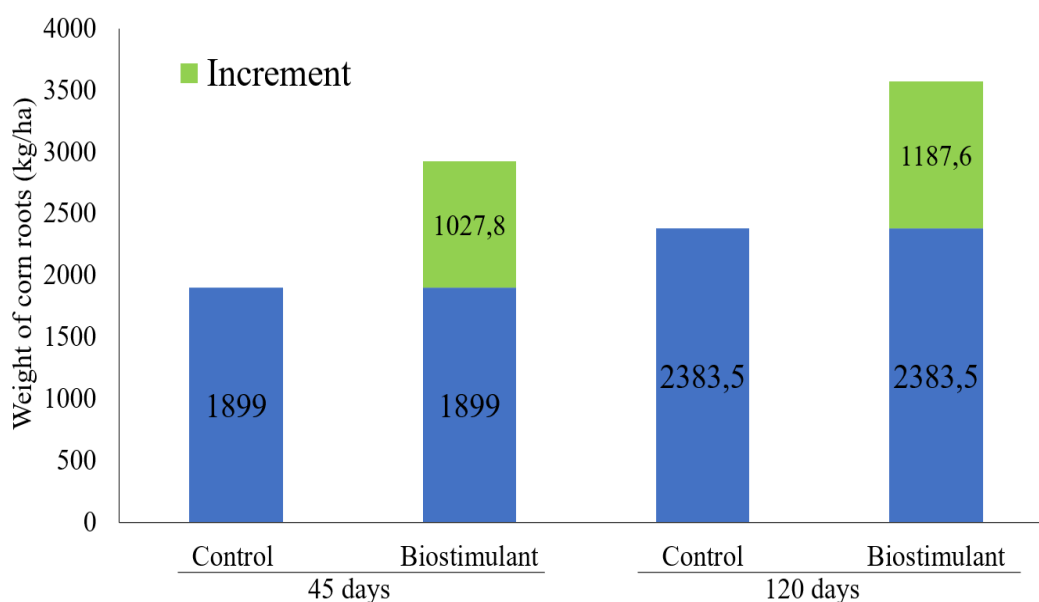
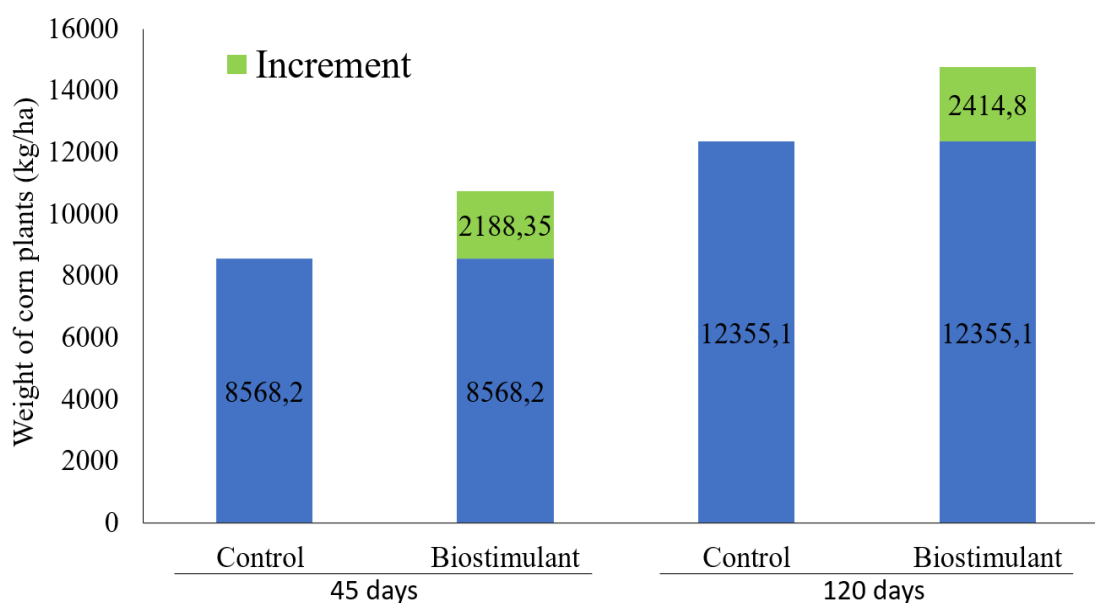


Figure 6. Weight of maize plants and increase in different treatments at 45 and 120 days after emergence. Considering 75,000 plants per hectare.



The increase in plant biomass is directly associated with greater carbon retention in plant tissues. The interaction between carbon emission (via soil respiration) and carbon retention (via plant growth and microbial biomass formation) provides the basis for estimating carbon balance in agricultural systems. For each cropping system, depending on soil microbial activity and the extent of plant biomass production, it is possible to estimate the relative efficiency of management practices and technological inputs in contributing to low-carbon agricultural strategies.

Carbon retention in maize plant tissues was estimated for the different treatments based on approaches reported in previous studies [20–24]. The growth stimulation observed under the biostimulant treatment resulted in greater carbon retention per unit area compared with the control. This increase in retained carbon reflects the higher biomass production observed in the treated system and suggests potential benefits for low-carbon agricultural management.

Total CO₂ emissions over the crop cycle were estimated for each treatment using mathematical models derived from the in vitro basal respiration tests together with in situ soil respiration measurements obtained during maize cultivation (Table 1). These estimates incorporate the temporal dynamics of microbial activity and soil respiration throughout the evaluation period.

Table 1. Total carbon dioxide (CO₂) emission, total carbon (C) retention, and net CO₂ emission balance in the maize crop under the control and biostimulant treatments.

Treatments	Total CO ₂ emission (kg/ha)*	Total Cretention in the plant (kg/ha)	Net CO ₂ emission balance (kg/ha)
Control	1027.82 b**	338.96 b	688.04 b
Biostimulant	1141.25 a	418.22 a	723.03 a
CV (%)	12.81	18.05	16.4

*Determined to use the CO₂ emission equations during the crop period.

**Media with the same letter in the columns did not reduce each other using the Tukey test at 5% probability. CV=coefficient of variation.

Data on total carbon retention per unit area (kg ha⁻¹) in maize plant tissues, combined with estimates of CO₂ emissions derived from microbial activity, allowed the calculation of the net carbon emission balance for the crop under the evaluated treatments (Table 1). To assess the efficiency of food production in terms of reduced atmospheric emissions, it is important to consider the relationship between the net CO₂ emission balance (kg ha⁻¹) and grain yield across treatments. This relationship provides an estimate of CO₂ emissions per unit of grain produced and can be used as an index for comparing management practices and technological inputs in terms of environmental performance. It represents a useful metric for evaluating production systems that aim to combine reduced environmental impact with satisfactory productivity.

Yield values for each treatment and the corresponding CO₂ emissions per unit of grain are presented in Table 2. As noted by García-Palacios and Chen [3], total greenhouse gas emissions associated with grain production depend on multiple factors, including soil type, temperature and moisture conditions during the crop cycle, organic matter content and decomposition rates, and the intensity of mechanization and management practices, among others.

However, considering only the balance between microbial activity in the soil and CO₂ emission and retention in the system, the values observed in this work corroborate data presented in specialized literature [25-29], presenting clear and consistent information on the contributions of the biostimulants SoilPlus® and FieldStim® to achieving greater agricultural and environmental sustainability.

Table 2. Maize productivity and CO₂ emissions per ton of grains under the control and biostimulant treatments.

Treatments	Net CO ₂ emission balance (kg/ha)	Productivity (kg/ha)	CO ₂ emission per Mg of grain (kg/ha)
Control	688.04 b*	8100.40 b	84.94 a
Biostimulant	723.03 a	9558.25 a	75.64 b
CV (%)	13.94	14.77	8.4

*Determined to use the CO₂ emission equations during the crop period.

**Media with the same letter in the columns did not reduce each other using the Tukey test at 5% probability. CV=coefficient of variation.

Beyond the specific conditions evaluated in this study, the results highlight the broader potential of microbial biostimulant-based strategies within climate-smart agricultural systems. Technologies capable of simultaneously enhancing soil biological activity, improving crop productivity, and increasing carbon retention in plant biomass may contribute to reducing the carbon intensity of food production at regional and global scales. If validated under diverse soil types, climates, and cropping systems,

such approaches could support the transition toward low-carbon agriculture by improving carbon-use efficiency per unit of yield and promoting greater integration between soil management and climate mitigation strategies. Future research should therefore focus on long-term and multi-site evaluations, the inclusion of additional greenhouse gases in carbon balance assessments, and the integration of these practices into broader climate-smart farming frameworks. Such efforts would help clarify the scalability of biologically based technologies and their role in supporting sustainable agricultural intensification under changing climatic conditions.

CONCLUSION

The results of this study demonstrate that the application of microbial biostimulants promoted measurable changes in soil biological activity, crop development, and carbon dynamics in Maize production systems. Biostimulant treatments stimulated microbial activity, as evidenced by increased β -glucosidase activity and greater microbial proliferation, indicating enhanced soil biological functioning and intensified carbon cycling processes.

Although biostimulant application resulted in higher basal soil respiration and increased total CO_2 emissions during the crop cycle, this response was accompanied by greater carbon retention in both microbial biomass and plant tissues. The stimulation of root and shoot biomass production led to higher carbon sequestration per unit area, suggesting that the increased carbon influx partially compensated for the higher efflux associated with enhanced microbial activity.

Importantly, when emissions were evaluated relative to crop productivity, the biostimulant treatment exhibited improved carbon-use efficiency, with lower CO_2 emissions per unit of grain produced compared with the control. This result indicates that productivity gains associated with microbial biostimulation may contribute to more sustainable production systems by reducing emission intensity per unit of output.

Overall, the findings suggest that microbial biostimulants may represent a promising approach for low-carbon agricultural systems by enhancing soil biological functioning, increasing biomass production, and promoting carbon retention in agroecosystems. Nevertheless, long-term and multi-site studies are necessary to more accurately quantify net carbon balances under different environmental conditions and management regimes, as well as to evaluate the scalability of these technologies within climate-smart agricultural frameworks.

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