

Influence of humic and fulvic substances on soil attributes

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Abstract

Humic and fulvic substances are organic compounds originating from the decomposition of plant and animal residues in the environment, which can be used as alternative inputs for the management of various crops. The objective of this work was to evaluate the influence of humic and fulvic substances on the chemical and microbiological attributes of the soil in soybean crops. Soil samples were collected at a depth of 0-10 cm before planting and at the flowering stage (R2), and chemical attributes were evaluated, such as macronutrient content, pH and organic matter, and microbiological attributes such as Microbial Biomass Carbon (MBC), Basal Respiration (BR), microbial and metabolic quotient to determine microbial activity. In agronomic parameters, plant development and productivity were evaluated. In the results of chemical attributes, little change was observed between treatments in the concentration of macronutrients; however, an improvement was observed in the concentration of organic matter in areas that received humic and fulvic substances at a dose of 4 L ha⁻¹, a fact also observed in microbiological attributes, with an increase in the microbial community and significant improvement in biological activity of the soil. Data reflected the increase in productivity, with an increase of 10 to 14 bags per hectare, without and with association with *Bacillus sp.* respectively. It is concluded that humic and fulvic acids at a dose of 4 L ha⁻¹ with or without association with the bacteria *Bacillus sp.* promoted improvements in the microbiological attributes of the soil and consequently in the development of soybean cultivation.

keywords:

Glycine max, bioinputs, rhizobacteria, soil quality bioindicators.



Introduction

Soybean [*Glycine max* (L.) Merrill] is currently considered one of the most important raw materials, both nationally and worldwide. This legume has a high nutritional value in its composition and stands out for having a high amount of protein in its grain, thus becoming an excellent option for replacing animal protein with one of vegetable origin in human nutrition (Lopes *et al.*, 2016).

The largest soybean production in Brazil is concentrated in the states of Mato Grosso (considered the largest Brazilian producer), Paraná, Rio Grande do Sul and Goiás. In the 2022-2023 harvest, Brazil had a production of 154 million tons of grains of soybeans (Conab, 2024). Like other important crops at an economic and social level, soybeans have different nutritional requirements for successful development. Nutritional deficiencies are responsible for reduced productivity and are associated with symptoms characteristic of the lack of each nutrient (Conab, 2016).

The appropriate use of fertilizers in soybean-producing regions is essential to achieve high productivity. The physical-chemical analysis of soils enables the correct supply of the main nutritional needs of the crop and aims to optimize the costs of implementing and maintaining the crop (Conab, 2016). Soybeans have different nutritional requirements throughout their development, in addition to the macronutrients (C, H, O) provided by the atmosphere (O_2 , CO_2 and H_2O), the crop also lacks nutrients that are provided by the soil, such as: P, K, Ca, Mg, S, B, Cl, Cu, Fe, Mn, Mo, Co and Zn and, in the case of N, partly through the soil and partly through the atmosphere.

Nutritional disorders are among the factors responsible for reduced productivity (Carmello and Oliveira, 2006). Knowing the importance of nutrients to plants and their effects on development and productivity, the search for stimulants that can contribute to better absorption of nutrients in the search for greater productivity has become the target of many studies. Biofertilizers, bioregulators, biostimulants and bioactivators are cited as the main stimulants capable of promoting important effects on plants, stimulating the soil microbial community in order to alter the development and productivity of crops (Morzelle *et al.*, 2017).

The use of these stimulants has become an important tool for producers and researchers in the search for increasing crop productivity, with less impact on the environment (Soares, 2013). Among the stimulants, biofertilizers stand out. Bearing in mind that the application of biofertilizers can contribute to better production of soybean crops, the search for information regarding the application via sowing furrows is necessary to provide knowledge of different management alternatives and use of these products, such as impact on soil microorganisms, responsible for the decomposition of organic matter, mineralization and solubilization of nutrients to the plant.

The objective of the work was to evaluate the influence of the application of humic and fulvic substances on the chemical and microbiological attributes of the soil in the development of soybean cultivation.

Materials and methods

Characterization of the experimental area

The experiment was carried out in the field at crop 2022-2023, in a typical Eutrophic Red Latossolo in the municipality of Itambaracá, Paraná State, Brazil, which is located at latitude $22^{\circ} 58' 16.2''$ S and longitude $50^{\circ} 28' 59.9''$ W, with an altitude of 420 m above the sea level, the climate classification is Cfa (Köppen and Geiger) humid subtropical, the area has a history of a system of lack of preparation for the cultivation of soybeans and corn, with the following chemical characteristics: organic material = 25.1 g kg^{-1} ; pH = 4.6; P = 14.9 mg dm^{-3} ; K = $0.6 \text{ mol}_c \text{ dm}^{-3}$; Ca = $5.8 \text{ mol}_c \text{ dm}^{-3}$; Mg $1.3 \text{ mol}_c \text{ dm}^{-3}$; H+Al = $5.1 \text{ mol}_c \text{ dm}^{-3}$; CTC = $12.8 \text{ mol}_c \text{ dm}^{-3}$; V = 59.7%. And microbiological characteristics: microbial biomass carbon (MBC) = $44.05 \text{ mg C kg}^{-1}$; basal respiration (RB) = $0.31 \text{ mg C-CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$; metabolic quotient (qCO_2) = 7.14; microbial quotient ($qMIC$) = 0.27%.

Experimental design

The experiment was carried out from October 2021 to March 2022 in a total area of 4 200 m². Treatments consist of [C]= control; [BAC]= *Bacillus* sp. isolated in concentration 10⁹ UFC mL⁻¹; [AHF1]= humic and fulvic 2 L ha⁻¹; [AHF2]= humic and fulvic 4 L ha⁻¹; [AHF1+BAC]= humic and fulvic 2 L ha⁻¹ + *Bacillus* sp.; [AHF2+BAC]= humic and fulvic 4 L ha⁻¹ + *Bacillus* sp., having plots with an area of 700 m² (10 m wide by 70 m long) distributed in the delineation of strip plots. In each plot, five soil samples were collected between the planting lines, using a screw auger at a depth of 0-10 cm for microbiological analyzes and 0-20 cm for chemical analyzes, with each sample consisting of 10 subsamples that were joined together. In a bucket and homogenized to remove each main sample.

The samples were stored in plastic bags and transported in a thermal box so as not to lose the characteristics of the soil collected, and to avoid any type of subsequent interference in the analyses. The samples were taken on the same day to the Soil Microbiology Laboratory, at UENP/CLM. Soybean sowing was carried out on 10-09-2021, with a spacing of 0.45 cm between rows. Base fertilizer of 250 kg ha⁻¹ of formula 02-20-18.

Soil collection for chemical and microbiological analyzes was carried out post-emergence at the full flowering stage (R2). In chemical assessments, analyzes were carried out for macronutrients, organic matter and pH. In the microbiological analyzes, microbial biomass carbon (MBC), basal respiration (BR), metabolic quotient (qCO_2) and Microbial quotient ($qMIC$) were evaluated.

The soybean harvest was carried out on 02-17-2022, the six areas were harvested, separated and weighed to obtain productivity results. At the time of harvest, the grains had 19% moisture, determined after collecting a 400 g portion and taken to the integrated cooperative to determine moisture and subsequently corrected to 14% moisture to determine the standard net marketing weight.

Chemical and microbiological attributes of the soil

Soil chemical analysis

drying the soil samples in the air, the pH was determined in 0.01 M CaCl₂, P, Ca²⁺, Mg²⁺, K⁺, and Al³⁺. The Ca²⁺, Mg²⁺ and Al³⁺ contents were extracted with 1 N KCl and determined by atomic absorption (Ca²⁺ and Mg²⁺) and titration with 0.025 M NaOH (Al³⁺); P and K⁺ were extracted with the Mehlich-1 extractor and determined by flame ionization spectrophotometry (K⁺) and the molybdenum blue method (P), according to Embrapa (2009).

Determination of organic matter

The organic matter content was determined due to the mass loss of the incinerated residue, considering the material lost through burning in the temperature range from 105 °C to 550 °C, according to Embrapa (2009); Tedesco (1995).

Determination of microbial carbon biomass (MBC)

Considered the living and most active part of organic matter. It is made up of fungi, bacteria and actinobacteria that act in processes ranging from soil formation to the decomposition of organic waste, nutrient cycling, bioremediation, among others. Soil microbial biomass carbon (MBC) was determined by the indirect fumigation-extraction (FEI) method proposed by Silva *et al.* (2007).

Determination of basal soil respiration (BR)

Basal respiration is the amount of CO₂ released by the respiration of microorganisms, a method used to evaluate the metabolic activity of the soil (Silva *et al.*, 2007).

Determination of the soil metabolic quotient (qCO_2)

The metabolic quotient (qCO_2) is the ratio between basal respiration and soil microbial biomass per unit of time (Anderson and Domsch, 1993).

Determination of the microbial quotient ($qMIC$)

The microbial quotient ($qMIC$) represents the ratio between microbial biomass carbon (MBC) and total organic carbon (TOC). This ratio used as an indicator of the quality of soil organic matter, indicating the amount of organic carbon that is immobilized in the biomass and demonstrating the efficiency of microorganisms in using organic compounds (Silva *et al.*, 2010).

Agronomic assessment

Agronomic evaluations were carried out during the development of the crop. At 07, 14 and 21 days after planting, the plant stand was evaluated. At the R2 vegetative stage, 10 plants were collected per plot to determine fresh and dry mass of shoots, roots and nodules. The plants were weighed *in situ* (shoot and root) to determine the fresh mass and to determine the dry mass, the plants were placed in a paper bag and dried in a forced air circulation oven at 60 °C, until obtaining constant weight. Productivity was determined by collecting plants in three rows of 1 m, totaling seven subsamples per plot.

Statistical analysis

The results of the chemical and microbiological attributes obtained were subjected to analysis of variance and the Tukey test was applied and the results of the agronomic parameters were subjected to the T test and the Scott-Knott test for productivity, to compare the means at the level of 5% probability, using the Sisvar software (Ferreira, 2019).

Results and discussion

In chemical parameters, the increase in organic matter content in the soil stands out in treatments with spray application in the total area of the plot of 4 L ha⁻¹ without and with *Bacillus* sp. inoculation (Table 1), this increase reflected an increase in microbial biomass without the inoculation of *Bacillus* sp. with the presence of the bacteria, it was noted that there was competition with the inoculated bacteria and the native biota; however, the biomass was greater than the control (Table 2).

Table 1. Chemical analysis carried out at the full flowering stage (R1-2) in soybean crops.

Treatment	MO (g kg ⁻¹)	pH ^(*) CaCl ₂	P ^(*) (mg dm ⁻³)	K ^(*)	Ca ^(*)	Mg ^(*)	H+Al	CTC ^(*)	V (%)
				(mol _e dm ⁻³)					
C	27.8 c	5.5	14.5	0.7	7.2	1.2	6.5 a	15.6	58.6 b
BAC	28.1 bc	5.2	12.6	0.7	7.4	1.3	5.9 ab	15.3	61.2 ab
AHF1	27.1 c	5.3	31.5	0.7	7.5	1.3	5.6 b	15.1	62.8 a
AHF2	29.8 ab	5.5	19	0.7	7.5	1.2	5.7 b	15.1	62.5 a
AHF1+BAC	28.9 bc	5.1	17.3	0.7	7.4	1.3	5.8 b	15.2	62 a
AHF2+BAC	30.8 a	5.2	25	0.7	7.6	1.3	6 ab	15.6	61.6 ab
CV (%)	3.55	5.17	59.28	32.71	3.05	4.7	4.94	2.37	2.56

OM= organic matter; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; H+Al= potential acidity; CTC= cation exchange capacity; V= base saturation; [C]= control; [BAC]= *Bacillus* sp.; [AHF1]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹; [AHF2]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹; [AHF1 +BAC]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹ + *Bacillus* sp.; [AHF2+BAC]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹ + *Bacillus* sp. Means followed by the same lowercase letter in the column do not differ from each other using the Tukey Test at 5% probability; *= there was no statistical difference between treatments.

Table 2. Analysis of the microbiological attributes of soils at the stage of full flowering of the soybean crop.

Trat	COT (g kg ⁻¹)	SMBC (mg C kg ⁻¹ soil)	BRS (mg C-CO ₂ kg ⁻¹ h ⁻¹)	qCO ₂ RBS/BMS	qMIC (%)
C	16.14 c	52.89 d	0.38 d	7.19 c	0.33 d
BAC	16.3 bc	94.92 c	1.03 b	11.84 a	0.53 c
AHF1	16.74 abc	133.35 b	0.99 bc	7.5 c	0.8 b
AHF2	17.85 a	176.01 a	1.36 a	7.71 bc	0.99 a
AHF1+BAC	15.71 c	137.11 b	1.26 a	9.22 b	0.87 ab
AHF2+BAC	17.3 ab	96.42 c	0.76 c	7.91 bc	0.56 c
CV (%)	3.55	7.79	12.19	10.29	9.19

Total organic carbon (TOC); soil microbial biomass carbon (SMBC); basal breathing (BR); soil metabolic quotient (qCO₂); microbial quotient (qMIC); [C]= control; [BAC]= *Bacillus* sp.; [AHF1]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹; [AHF2]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹; [AHF1+BAC]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹ + *Bacillus* sp.; [AHF2+BAC]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹ + *Bacillus* sp. Means followed by the same lowercase letter in the column do not differ from each other using the Tukey test at 5% probability.

The other chemical parameters are within good soil management for soybean cultivation. However, comparing with the initial soil analysis and the analysis at full flowering, a considerable improvement in soil fertility is observed with the use of substances based on humic and fulvic acids, an improvement in CEC and in saturation of bases. The effects of applying humic substances on plant production and nutrient absorption depend on the source of origin of the humic and fulvic substances and their concentration. In high concentrations of humic acids, more stress-tolerant, productive and healthy plants develop (Ampong *et al.*, 2022).

In view of the results obtained with the chemical analysis of the soil at the R2 stage of soybeans, a difference was found in the P content (melich⁻¹ mg dm⁻³) in the soil, resulting from the application of humic and fulvic substances. The increase found in the level of P available in the soil may have occurred as a result of the ability of humic and fulvic substances to act as complexing agents. According to Caron *et al.* (2015), humic and fulvic substances can promote an increase in the soluble phosphorus content through the complexation of Fe⁺² and Al⁺³ (acidic soils) and Ca⁺² (alkaline soils).

With the possible use of biofertilizers based on humic and fulvic acids in the long term, it is expected that increases in the levels of other elements in the soil will be observed. Li *et al.* (2019), evaluated the effects of applying humic and fulvic acids under continuous peanut cultivation in three consecutive years, found increases in total and available N, P and K contents and in organic matter contents, verifying the maximum effect in the third year of application of humic and fulvic substances.

Regarding microbiological attributes, it was observed that all treatments showed an increase in soil microbial biomass (MBC), demonstrating an increase in the microbial population. The use of humic and fulvic substances combined or not with bacteria did not cause metabolic stress (qCO₂), remaining at the same levels as the control (7.19), which shows an increase only in the application of the bacteria *Bacillus* sp., the increase in the metabolic quotient (11.84) (Table 2).

The microbial activity (qMIC) evaluated in the test demonstrated an improvement in microbial activity in decomposing and mineralizing soil organic matter in all treatments, with emphasis on the AHF2 treatment (0.99). The functional groups of humic and fulvic substances favor the growth and increase in plant biomass, as well as an increase in the number of fruits, resulting in an increase in crop productivity (Halpern *et al.*, 2015).

This improvement in microbiological attributes when using rhizobacteria and especially substances based on humic and fulvic acids at doses of 2 and 4 L ha⁻¹ combined or not with *Bacillus* sp. demonstrated an increase in microbial population and activity in the soil (Table 2), when compared to initial conditions before planting.

The improvement in microbiological conditions of the soil and in organic matter of soil, reflected in agronomic parameters of soybean plant, demonstrating an increase in dry mass of shoot in treatments with humic and fulvic substances in the two doses evaluated combined with *Bacillus* sp. (13.1% and 18.42% respectively), despite being statistically equal to the control (Table 3).

Table 3. Agronomic parameters of the soybean plant (shoot, root, nodules and productivity).

Trat	DMS (g)	DMR (g)	MNOD (g)	Productivity	
				(kg ha ⁻¹)	(Saca ha ⁻¹)
C	21.22 ab	2.6 ab	0.19 bc	3 523.37 b	58.72
BAC	20.18 b	2.71 a	0.28 a	3 286.22 b	54.77
AHF1	21.35 ab	2.23 b	0.2 bc	3 704.06 b	61.73
AHF2	22.29 ab	2.31 ab	0.17 c	4 133.19 a	68.89
AHF1+BAC	24.06 ab	2.57 ab	0.25 ab	3 478.2 b	57.97
AHF2+BAC	25.13 a	2.3 ab	0.15 c	4 381.63 a	73.03
CV (%)	18.64	16.19	34.71	16.5	-

DMS= dry mass of the shoot; DMR= dry mass root; MNOD= mass of nodules; C= control; BAC= *Bacillus* sp.; [AHF1]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹; [AHF2]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹; [AHF1+BAC]= substances based on humic and fulvic acids at a dose of 2 L ha⁻¹ + *Bacillus* sp.; [AHF2+BAC]= substances based on humic and fulvic acids at a dose of 4 L ha⁻¹ + *Bacillus* sp. Means followed by the same lowercase letter in the column do not differ from each other using the T Test at 5% probability for agronomic parameters (DMS, DMR, MNOD) and Scott Knott for productivity.

Leonardite derivatives altered the microbiome structure of soil grown under greenhouse conditions. Plants treated with humic acids showed greater microbial diversity and richness, with a predominance of proteobacteria, causing a beneficial impact on plant growth (Hita *et al.*, 2020). Inoculation of plant growth-promoting bacteria (PGPB) and humic acids regulate genes related to plant protection and oxidative stress. These adaptive metabolic changes can reduce plant stress under biotic and abiotic stress conditions (Galambos *et al.*, 2020).

This combination is beneficial as it induces secondary protection and promotes plant growth. Prolonged use of humic acids in agriculture alters soil enzymatic activity and structure of soil microbial community, increasing the population of beneficial bacteria and fungi (Li *et al.*, 2019). Plants trigger production of phytohormones, causing changes in root and shoot growth, and rhizodeposition that will then influence the microbiota in the community composition of the soil-plant system.

In the root parameter, no major variations in dry weight of root were observed, a fact explained by the extreme difficulty in removing root from clayey soil, however, a significant difference was observed between the BAC and AHF1 treatments. The treatment with bacteria showing higher dry mass may be related to the higher dry mass of the nodules (Table 3). However, the greater nodulation was not reflected in productivity (54.7 bags ha⁻¹).

The highest productivity was observed in treatments with humic and fulvic substances at a dose of 4 L ha⁻¹ with and without *Bacillus* sp. (4 381.63 and 4 133.19 kg ha⁻¹ respectively), with an increase of 14 and 10 bags ha⁻¹ respectively compared to the control (Table 3). Caron *et al.* (2015) stated that humic substances can be used as inputs, with the aim of positively conditioning the soil, improving development of crops where it is used, mainly root system.

These same authors report that humic substances can cause beneficial effects such as greater root growth, greater effectiveness in nutrient absorption and productivity in the aerial part of the plant through processes such as hormonal signaling and metabolic changes (Caron *et al.*, 2015).

Plant nutrition, as well as soil fertility, stands out among the factors that are directly related to successful cultivation and productivity and constitute the management of nutrients considered essential for plant growth. The nutritional requirements of soybeans and the crop's export potential

are characteristics determined by genetic factors, but influenced by climatic factors, soil fertility and cultural management (Oliveira *et al.*, 2019).

Biofertilizers, bioregulators, biostimulants and bioactivators are cited as the main stimulants capable of promoting important effects on plants, stimulating the soil microbial community in order to alter the development and productivity of crops (Morzelle *et al.*, 2017).

Another way of including stimulants in crop management mentioned in some studies is foliar application. According to Bertolin *et al.* (2010), the use of these products in soybean cultivation provides an increase in the number of pods per plant and grain productivity both when applied through seed treatment and foliar application. Another way of inserting stimulants in soybean crop management is application via sowing furrow, however the subject is discussed less frequently in literature when compared to seed treatment and foliar application.

Humic acid-based products can increase the nutrient retention capacity of the soil, improving nutrient cycling within different compartments of soil organic matter, as well as the exchange of nutrients dissolved in water in soil pores (Ampong *et al.*, 2022).

As organic additives can act as slow-release fertilizers, this beneficial situation could be maintained for long periods of time, in contrast to mineral fertilizers alone. The combined use of humic acid and inorganic fertilizers increases long-term plant yield and quality (Li *et al.*, 2019). Humic acids alleviate the problems associated with the continuous cultivation system. In the presence of these, it increases the levels of NPK available to plants, and organic matter in the soil, resulting in greater absorption of NPK by the plant.

Furthermore, improvements in the physicochemical quality of the soil lead to plant growth by improving the microbial diversity and enzymatic activities of the soil (Li *et al.*, 2019). The importance of using substances based on humic and fulvic acids applied via soil to improve microbiota and consequently, physical and chemical properties are highlighted.

Conclusions

Humic and fulvic substances positively influenced the chemical and microbiological attributes evaluated, mainly observing the improvement in the organic matter content, a fact that directly influenced the biomass and microbial activity of the soil, and consequently the decomposition and mineralization of organic matter, improving the efficiency in making nutrients available to the plant, a fact that is reflected in the productivity of soybean crops.

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