



## Mining waste associated with bioinoculants and cattle manure for the mineral nutrition and growth of maize<sup>1</sup>

### Resíduo de mineração associado com bioinoculantes e esterco bovino na nutrição mineral e crescimento do milho

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#### HIGHLIGHTS:

*Waste from vermiculite extraction improves soil chemical and biological attributes.*

*Cattle manure and beneficial microorganisms increase nutrient release rate from vermiculite waste.*

*Vermiculite residue can be a complementary source of nutrients for maize crop.*

**ABSTRACT:** Vermiculite mining residues, although of low solubility, contain nutrients such as calcium, magnesium, and potassium at concentrations that allow their agricultural use. Beneficial bacteria can increase the solubility of this waste and its efficiency. The objective of the present study was to evaluate the influence of bio-inoculation of vermiculite residue, associated with cattle manure, on the release of nutrients and initial growth of maize. A completely randomized design was performed in a greenhouse with seven treatments and four replications. The treatments were: T1 - soil without fertilization; T2 - soil fertilized with soluble sources of nutrients (conventional fertilization); T3 - application of vermiculite residue (VR); T4 - VR enriched with cattle manure (CM); T5 - VR + CM with *Bacillus subtilis*; T6 - VR + CM + *B. amyloliquefaciens*, and T7 - VR + CM + *B. subtilis* + *B. amyloliquefaciens*. Regardless of the use of cattle manure or beneficial microorganisms, vermiculite mining waste increased the availability of Ca and K in the soil, but was not able to match the soluble sources of nutrients. Mining waste associated with cattle manure and beneficial microorganisms (*B. subtilis* and *B. amyloliquefaciens*) increased microbial activity and enhanced the release of available calcium for maize. VR increased the growth and the dry mass production of maize compared to the non-fertilized soil, but was consistently inferior to conventional fertilizer.

**Key words:** beneficial bacteria, vermiculite residue, remineralizers, solubilizers

**RESUMO:** Os resíduos da mineração de vermiculita, embora de baixa solubilidade, contêm nutrientes como cálcio, magnésio e potássio em concentrações que permitem seu uso agrícola. Bactérias benéficas podem aumentar a solubilidade deste resíduo e sua eficiência. Objetivou-se avaliar a influência da bioinoculação do resíduo de vermiculita, associado ao esterco bovino, na liberação de nutrientes e no crescimento inicial do milho. Foi realizado delineamento inteiramente casualizado em casa de vegetação com sete tratamentos e quatro repetições. Os tratamentos foram: T1 - solo sem adubação; T2 - solo adubado com fontes solúveis de nutrientes (fertilização convencional); T3 - aplicação de resíduo de vermiculita (VR); T4 - VR enriquecido com esterco bovino (CM); T5 - VR+CM com *Bacillus subtilis*; T6 - VR + CM + *B. amyloliquefaciens* e T7 - VR + CM + *B. subtilis* + *B. amyloliquefaciens*. O VR aumentou a disponibilidade de Ca e K no solo, porém seu desempenho foi inferior às fontes solúveis. Independentemente da utilização de esterco bovino ou de microrganismos benéficos, os resíduos da mineração de vermiculita aumentaram a disponibilidade de Ca e K no solo, mas não foram capazes de igualar as fontes solúveis de nutrientes. Os resíduos de mineração associados ao esterco bovino e aos microrganismos benéficos (*B. subtilis* e *B. amyloliquefaciens*) aumentaram a atividade microbiana e aumentaram a liberação de cálcio disponível para o milho. Os resíduos da mineração de vermiculita aumentaram o crescimento e a produção de massa seca do milho em comparação ao solo não fertilizado, mas foram consistentemente inferiores ao fertilizante convencional.

**Palavras-chave:** bactérias benéficas, resíduo de vermiculita, remineralizadores, solubilizadores

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## INTRODUCTION

Brazilian agricultural production, especially of commodities such as soybean and maize, is very dependent on the use of synthetic mineral fertilizers, almost all of which are imported from countries such as Canada, Russia, Ukraine, and Morocco (Benites et al., 2022). Although the production and use of these fertilizers are necessary for agricultural production, the raw materials used, in addition to negatively impacting the environment, are not renewable, so they have a determined period to end (Santos et al., 2016; Manning, 2018).

The agronomic use of industrial waste, such as that generated by vermiculite mining, can reduce part of the impacts generated by these activities (Marcelino et al., 2022). Brazil accounts for around 14% of global vermiculite production and generates around 30,000 tons of unused waste (Rojas-Ramírez et al., 2019). Vermiculite residues have nutrients such as K, Ca, and Mg in their composition, which are part of the structure of silicate minerals such as vermiculite, muscovite, and biotite (Silva et al., 2021; Marcelino et al., 2022). Increasing the rate of nutrient release by mining waste can be achieved by using rock mineral solubilizing microorganisms (Vasanthi et al., 2018; Fomina & Skorochod, 2020).

Among the technologies employed to solubilize minerals, the use of microorganisms, such as plant growth-promoting bacteria, is preferable since it promotes the solubilization of minerals from rocks containing plant nutrients (Etesami et al., 2017; Vasanthi et al., 2018; Fomina & Skorochod, 2020).

Growth-promoting bacteria can also synthesize organic substances (such as hormones, organic acids, and amino acids), capable of stimulating plant growth, and their use is an environmentally friendly technology (Rosa et al., 2020). The objective of present study was to evaluate the influence of bio-inoculation of vermiculite residue, associated with cattle manure, on the release of nutrients and initial growth of maize.

## MATERIAL AND METHODS

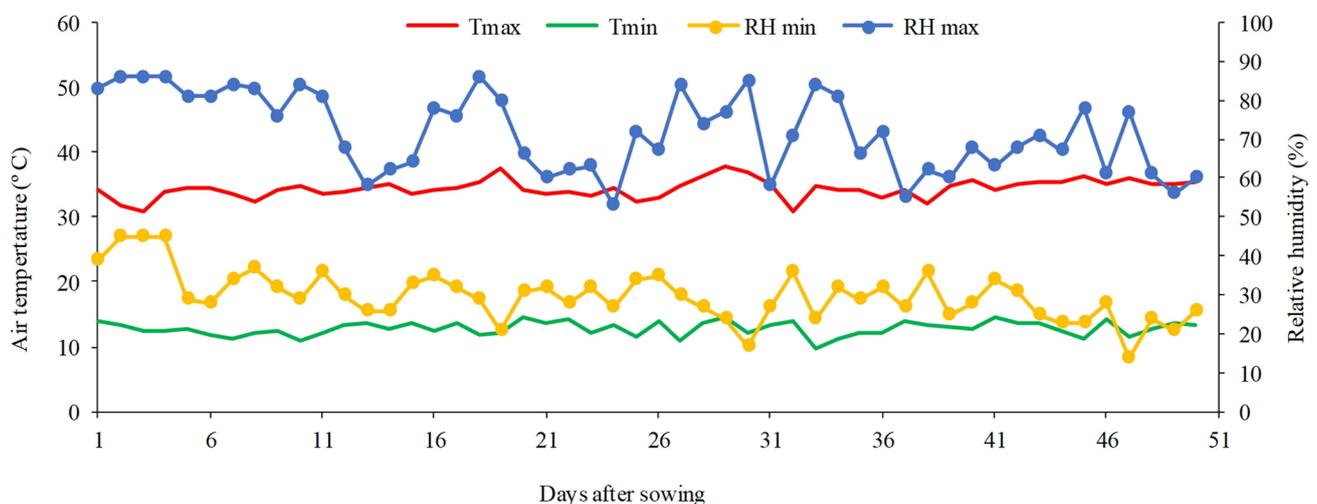
The study was performed from June to September 2022 under greenhouse conditions at the Center of Science and

Agri-Food Technology of the Universidade Federal de Campina Grande (CCTA/UFCG), Pombal, PB, Brazil (37° 48' 07" W, 6° 46' 12" S and 184 m of altitude). Maximum and minimum temperature and relative humidity data, inside the greenhouse, were measured during the experimental period (Figure 1).

The experiment was set up in a completely randomized design, with seven treatments and four replicates. Each replicate consisted of a single maize plant grown in a pot with 10 dm<sup>3</sup> of soil. The treatments (T) were constituted as follows: T1- soil without fertilization; T2 - soil fertilized with soluble sources of macro and micronutrients (conventional fertilization); T3 - application of vermiculite residue (VR); T4 - VR enriched with cattle manure (CM); T5 - VR+CM with *Bacillus subtilis*; T6 - VR+CM+ *B. amyloliquefaciens* and T7 - VR+CM+ *B. subtilis* + *B. amyloliquefaciens*.

Treatment T2 was established according to Novais et al. (1991), with the following doses in mg dm<sup>-3</sup>: N = 250; P = 200; K = 300; Ca = 200; Mg = 50; S = 50; B = 0.5; Cu = 1.5; Fe = 5; Mn = 4; Mo = 0.15 and Zn = 5.0. The macronutrient sources used were: urea [CO(NH<sub>2</sub>)<sub>2</sub>], single superphosphate (18% P<sub>2</sub>O<sub>5</sub>), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) analytical reagent (A.R.), and potassium chloride (KCl) (A.R.). For micronutrients, the following sources were used: copper sulfate (CuSO<sub>4</sub>.5H<sub>2</sub>O) (A.R.), manganese chloride (MnCl<sub>2</sub>.4H<sub>2</sub>O) (A.R.), zinc chloride (ZnSO<sub>4</sub>.7H<sub>2</sub>O) (A.R.), boric acid (H<sub>3</sub>BO<sub>3</sub>) (A.R.), and ammonium molybdate [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O] (A.R.). The treatments from T3 to T7 corresponded to the association of vermiculite residue with cattle manure and/or beneficial bacteria (*Bacillus subtilis* and *B. amyloliquefaciens*) according to the treatments.

The mineral residue of vermiculite was obtained from a disposal area of the União Brasileira de Mineração (UBM) mining company located in Santa Luzia, Paraíba state, Brazil. In the collected waste, the total contents of K, Na, P, Ca, and Mg were determined (Table 1). To determine the total contents, the vermiculite residue was crushed and passed through a 1.0 mm mesh sieve. Then, 4 mL of the aqua regia extractant (3 mL of conc. HNO<sub>3</sub> + 1 mL of conc. HCl) was added to 0.5 g of vermiculite residue according to McGrath



**Figure 1.** Maximum (Tmax) and minimum (Tmin) temperature, and maximum (RHmax) and minimum (RHmin) relative humidity of air during the field experiment period

**Table 1.** Composition of vermiculite waste and cattle manure used in the experiment

Material	C	N	P	K	Ca	Mg	Na	C/N
	(g kg <sup>-1</sup> )							
Vermiculite waste	ND	ND	0.40	25.95	30.07	69.28	2.31	-
Cattle manure	157.90	16.70	4.15	8.13	ND	ND	3.19	9/1

ND - Not determined

& Cunliffe (1985). Cattle manure was dried in an oven with forced air circulation at 60-65 °C, homogenized, ground in a mortar, passed through a 1.0 mm mesh sieve, and then analyzed for C, N, K, Na, and P (Table 1) according to Tedesco et al. (1995).

In the composition of treatments from T3 to T7, 200 g of vermiculite residue (80%) enriched with 50 g of cattle manure (20%) were used, totaling 250 g 10 dm<sup>-3</sup> of soil. This proportion was defined based on the potassium rate applied in T2 (300 mg dm<sup>-3</sup>) and the K concentrations contained in mining waste and cattle manure. For this, 100% of the K contained in cattle manure and 50% of the K contained in the vermiculite residue were considered. As a source of microorganisms for the composition of treatments T5, T6, and T7, commercial products containing *Bacillus subtilis* (BV-09, 1.0 x 10<sup>8</sup> CFU mL<sup>-1</sup>) and *Bacillus amyloliquefaciens* (1.0 x 10<sup>9</sup> CFU mL<sup>-1</sup>) were used. Before application, these products were diluted in the proportion of 1 mL 100 mL<sup>-1</sup> in sterilized distilled water, using previously sterilized glassware. The addition of these biological products to the other components of the treatments was carried out at the time of application of the treatments in the pots, at a dose of 10 mL per pot. This dose was based on Rocha et al. (2023) and the manufacturer's recommendation, considering a population of 50,000 plants per ha. After applying the treatments, soil moisture was adjusted to 60% of field capacity.

For the installation of the experiment, samples of a Argid (USDA, 2014) were used, which were randomly obtained in the 0 - 30 cm layer, collected five km away from the municipality of São Domingos, Paraíba, Brazil (37° 53' 09" W, 6° 50' 04" S and 211 m of altitude). After air drying, the samples were sent to the Soil and Plant Nutrition Laboratory of the CCTA/UFCG and passed through a 2.0 mm mesh sieve to carry out the chemical and physical characterization (Table 2) according to the methodologies proposed by EMBRAPA (2011). After the treatments were applied, the soil was incubated for 30 days with moisture corresponding to 60% of field capacity. The field capacity of the soil was determined based on the volume of water drained after adding 150 mL of water to 200 cm<sup>3</sup> of soil in a glass percolation tube, according to Ribeiro et al. (2022). Moisture control was carried out by daily weighing on a digital scale and complementation of the evaporated water to maintain the initial soil moisture. After the incubation

period, five seeds per pot of the hybrid maize cultivar K9555VIP3 were sown. This cultivar was chosen because it is demanding in terms of nutrients and fast-growing, besides having high yield and resistance to fall armyworm. Five days after emergence, thinning was performed, leaving a single plant per pot. During the experiment, it was not necessary to apply any phytosanitary product to control pests or diseases. The few weeds that appeared were pulled out manually and incorporated into the pot itself.

At 50 days after the emergence (V12 stage) of maize seedlings, plant height and culm diameter were evaluated. Culm diameter was evaluated at 5 cm from the plant collar. The following day, the plants were separated into leaves, culms, and roots and subsequently dried in an oven with forced air circulation at 65 ± 5°C to obtain the dry mass of leaves, culms, and roots and, by the sum, the total dry mass. In these tissues, the levels of N, P, and K were determined according to Tedesco et al. (1995) and their respective amounts accumulated in the plants, based on the dry mass produced. At the time of plant collection, a sample of about 300 g of soil was taken from each pot, for the evaluation of exchangeable calcium and magnesium, available phosphorus and exchangeable potassium, electrical conductivity of the extract (soil:water, 1:5 v:v) and pH in CaCl<sub>2</sub> according to EMBRAPA (2011).

The remaining soil samples from each plot were frozen for later evaluation of soil respiration rate (Soil Resp), microbial biomass carbon (C-mic), and metabolic quotient (Soil resp/C-mic). Soil microbial respiration was measured by capturing the C-CO<sub>2</sub> produced in the soil by NaOH (sodium hydroxide) in a hermetically closed environment (Alef & Nannipieri, 1995). Biomass carbon was measured using the irradiation/extraction method, whose basic principle is the elimination of microorganisms by electromagnetic irradiation in a microwave oven, and subsequently determining the carbon content in irradiated and non-irradiated samples. The carbon content in the soil samples was determined by the oxidation method with a K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution (potassium dichromate) at 0.066 mol L<sup>-1</sup> (Islam & Weil, 1998; Ferreira et al., 1999). Quantification of C-mic was carried out by titration of the remaining carbon, after the oxidation process with 0.03 mol L<sup>-1</sup> ammonium ferrous sulfate.

Nutrient use efficiency for N, P, and K was estimated based on the production of total dry mass (TDM) and the

**Table 2.** Physical and chemical attributes of soil (0 - 30 cm depth) used in the experiment

pH	EC <sub>1:5</sub>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	H + Al <sup>3+</sup>	Al <sup>3+</sup>	P	V
CaCl <sub>2</sub>	(dS m <sup>-1</sup> )	(cmol <sub>c</sub> dm <sup>-3</sup> )					(mg kg <sup>-1</sup> )		(%)
5.42	0.02	4.10	3.10	0.66	0.28	2.15	0.50	5.9	3.04
SOM	BD	PD	Sand	Silt	Clay	FC	PWP	AWC	TP
(g kg <sup>-1</sup> )	(g cm <sup>-3</sup> )		(%)						
7.92	1.45	2.67	638	145	217	15.7	6.45	9.25	45.7

SOM - Soil organic matter, V - Base saturation, BD - Bulk density, PD - Soil particle density, TP - Total porosity, FC - Field capacity, PWP - Permanent wilting point, AWC - Available water content

accumulated amount of each nutrient (AN), using the following equation: Nutrient use efficiency =  $(TDM)^2/AN$  (Siddiqi & Glass, 1981).

Statistical analysis was performed using analysis of variance (ANOVA) and the Scott-Knott means comparison test. All tests were performed at  $p \leq 0.05$  and  $0.01$  probability levels using SISVAR software (Ferreira, 2019). Before performing the analysis of variance and test of means, the data were subjected to the normality test using the Shapiro-Wilk test.

A multivariate analysis of variance (MANOVA), using the Hotelling test (Hotelling et al., 1947), was performed using principal components analysis (PCA), from a completely randomized multivariate model, in which the correlation between 14 selected variables was performed, using the PAST statistical analysis system (Hammer et al., 2001).

## RESULTS AND DISCUSSION

Treatments influenced ( $p \leq 0.01$ ) all variables evaluated in soil (soil pH, electrical conductivity, available phosphorus, calcium, magnesium, and K in the soil, soil respiration, biomass carbon, metabolic quotient ( $qCO_2$ )) and in maize plants (plant height, culm diameter, leaf dry mass, culm dry mass, total dry mass, accumulation of nitrogen, phosphorus, and potassium, use efficiency of nitrogen, phosphorus and potassium) (Table 3).

The treatments consisting of soluble sources (T2), mining waste enriched or not with cattle manure (T3 and T4) and mining residue associated with cattle manure and beneficial microorganisms (T5, T6 and T7) decreased the pH (Figure 2A) and increased the electrical conductivity (EC) of the soil (Figure 2B), the available phosphorus (P) contents (Figure 2C), and the exchangeable potassium (K) and calcium (Ca) (Figures 2D and 2F). Exchangeable magnesium (Mg) was not altered by the treatments (Figure 2E). The increases in EC, Ca, and K contents are indicators of the release of nutrients from the mining waste added in treatments T5, T6, and T7 (Silva et al., 2021; Marcelino et al., 2022). The increases in EC, Ca and soil respiration (Figures 2B, 2D and 2G) due to treatments T5, T6, and T7 are possibly due to beneficial microorganisms, which

promoted an increase in the solubility of nutrients present in the vermiculite residue (Etesami et al., 2017; Formina & Skorochod, 2020).

The increase in Ca concentration in the soil due to the addition of vermiculite residue, especially when inoculated with solubilizing bacteria, becomes even more relevant in soils with a high degree of weathering, acidic or sandy, where this nutrient is generally scarce. Beneficial microorganisms such as *B. subtilis* and *B. amyloliquefaciens* can produce acidic secondary metabolites, such as organic acids, which can contribute to the solubilization of nutrients such as Ca and K present in silicate minerals such as micas, vermiculite, biotite, and feldspars (Etesami et al., 2017; Rosa, 2020). In addition, these microorganisms can increase the diversity of others microorganisms involved in the decomposition and mineralization of organic matter (Huang et al., 2020; Lopes et al., 2020). In this context, soil respiration, which is a measure of soil microbiological activity, increased due to treatments T5, T6, and T7 (Figure 2G). The lower respiration rate in treatment T6 compared to treatments T5 and T7 is justified by the fact that different strains of beneficial bacteria affect soil respiration with different intensity, according to their interactions with soil attributes (Rao et al., 2021; Rocha et al., 2023).

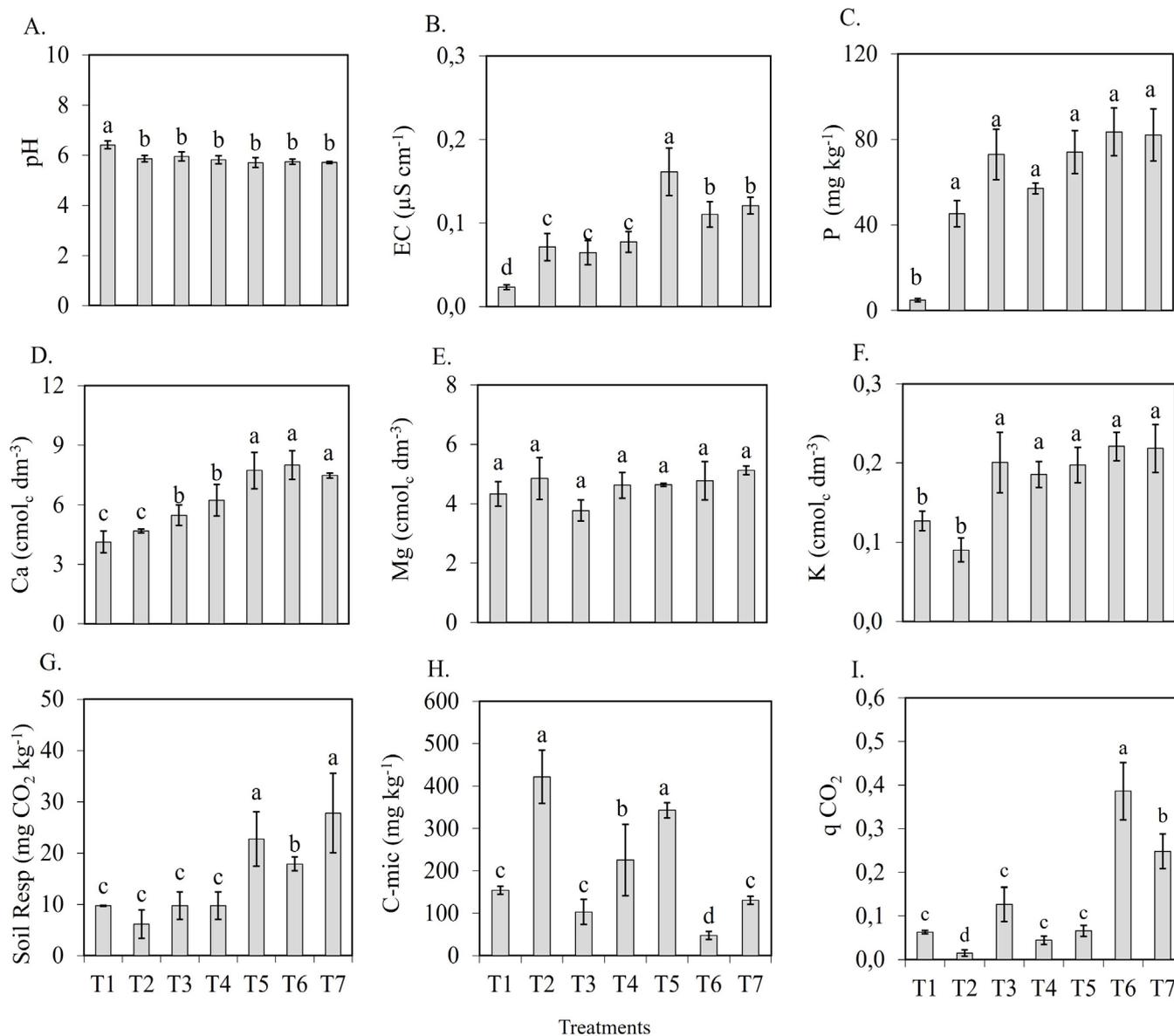
Treatments T2, T4, and T5 promoted the highest values of carbon in the microbial biomass (Figure 2H), while treatments T6 and T7 promoted the highest values of the metabolic quotient (Figure 2I). The higher carbon content of the microbial biomass does not always correlate positively with the respiration rate, due to the complexity of the interactions that occur between populations of microorganisms in the soil in the rhizosphere region (Ferreira et al., 1999). The increase in the metabolic quotient, in turn, represents an increase in soil oxygen demand due to the decomposition of organic matter (Rao et al., 2021) and possibly the solubilization of minerals present in the mining waste. On the other hand, the addition of *B. subtilis* (T5 and T7 treatments) diminished this effect.

The T2 treatment (soluble sources) promoted higher values for plant height (Figure 3A), culm diameter (Figure 3B), leaf dry mass (Figure 3C), culm dry mass (Figure 3D), and total dry mass (Figure 3E). For these variables, treatments T3, T4, T5,

**Table 3.** Summary of analysis of variance for soil pH, electrical conductivity (EC), available phosphorus (P), calcium (Ca), magnesium (Mg), and K in the soil, soil respiration (Soil resp), microbial biomass carbon (C-mic), metabolic quotient ( $qCO_2$ ), plant height (H), culm diameter (SD), leaf dry mass (LDM), culm dry mass (SDM), total dry mass (TDM), accumulation of nitrogen (N-plant), phosphorus (P-plant), and potassium (K-plant), use efficiency of nitrogen (NUE), phosphorus (PUE) and potassium (KUE)

SV	DF	Mean squares and F test						
		Soil pH	EC	P-soil	Ca-soil	Mg-soil	K-soil	Soil Resp
Treatments	6	0.246**	8041.975**	3116.230**	9.557**	0.747**	0.010**	2.630**
Error	21	0.022	475.027	79.508	0.372	0.202	0.000	0.160
CV (%)	-	2.50	24.23	14.87	9.78	9.81	13.12	26.93
		C-mic	$qCO_2$	H	SD	LDM	SDM	TDM
Treatments	6	731.078**	0.069**	2858.056**	129.804**	1060.855**	1861.301**	9153.902**
Error	21	17.896	0.002	34.601	4.43	2.533	3.684	24.759**
CV (%)	-	20.77	34.22	6.96	11.38	9.21	15.29	10.36
		N-plant	P-plant	K-plant	NUE	PUE	KUE	
Treatments	6	891753.52**	17662.034**	360464.911**	93.581**	4822.734**	233.267**	
Error	21	1786.340	70.130	969.493	1.450	56.452	1.687	
CV (%)	-	13.80	11.00	9.59	13.77	24.58	18.17	

SV - source of variation; CV - Coefficient of variation; \*\* $p \leq 0.01$  and \* $p \leq 0.05$  by F test; ns - non-significant data at  $p \leq 0.05$ ; DF - Degrees of freedom



T1- Soil without fertilization; T2 - Soil fertilized with soluble sources; T3 - Application of vermiculite residue (VR); T4 - VR enriched with cattle manure (CM); T5 - VR+CM with *Bacillus subtilis*; T6 - VR+CM+ *B. amyloliquefaciens* and T7 - VR+CM+ *B. subtilis* + *B. amyloliquefaciens*). Data are means  $\pm$  S.E. Lowercase letters compare the treatments by the Scott-Knott test at 0.05 significance level

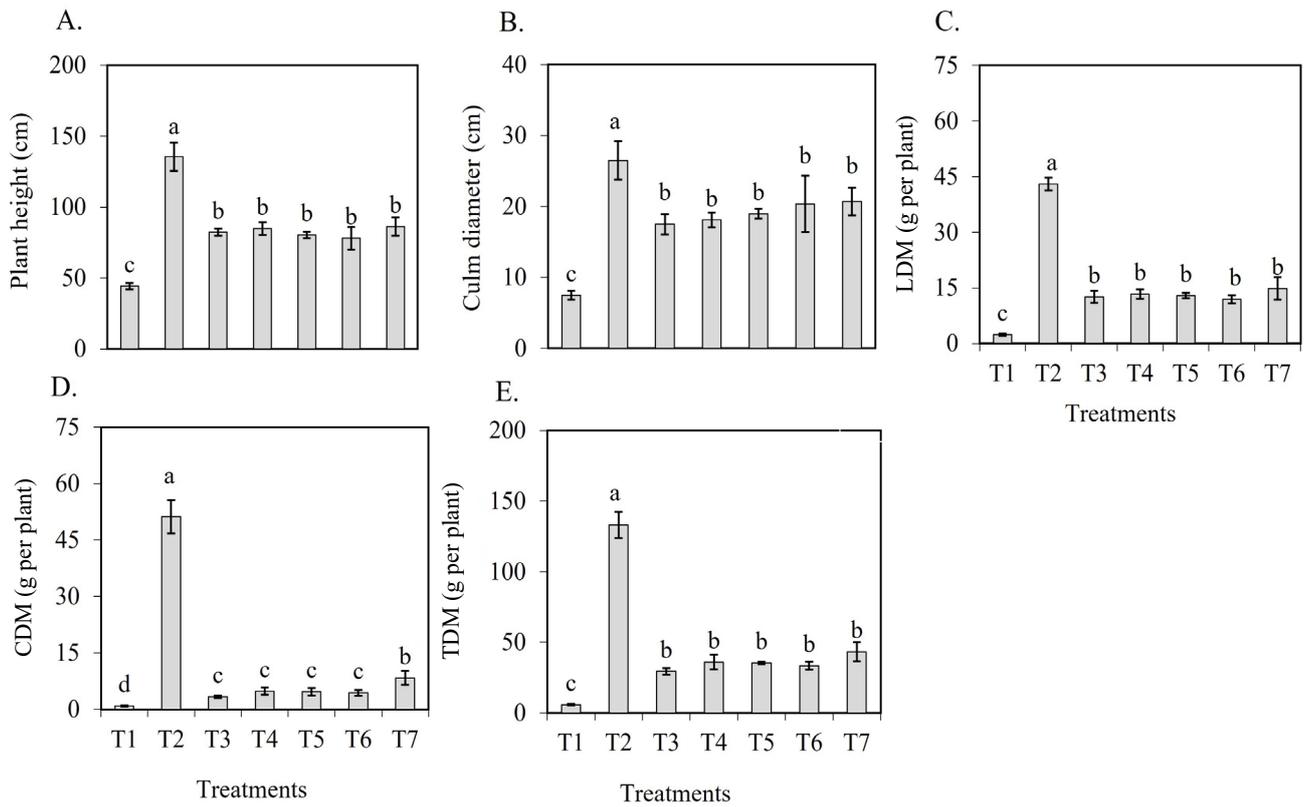
**Figure 2.** pH (A), electrical conductivity 1:5 (B), available phosphorus-P (C), exchangeable calcium-Ca (D), magnesium-Mg (E), potassium-K (F), soil respiration-Soil resp (G), carbon content of microbial biomass-C-mic (H), and metabolic quotient- $qCO_2$  (I) as a function of the treatments

T6, and T7 were superior to the control treatment (T1 - without fertilization), reaching, on average, 61% of the plant height and 72% of the culm diameter values obtained in the treatment with soluble sources (T2). The treatment containing cattle manure combined with the two strains of bacteria (T7) provided greater production of culm dry mass (SDM). Treatments T3, T4, T5, T6, and T7 provided similar effects for plant height, culm diameter, leaf dry mass, and total dry mass (Figures 3A, 3B and 3E). Treatments T3, T4, T5, T6, and T7, on average, promoted 26% of the total dry mass obtained in treatment T2, but promoted an increase in this variable of almost five times compared to the control treatment (Figure 3E).

These results demonstrated that the vermiculite mining residue, associated with organic matter and/or beneficial microorganisms, was not able to fully replace the soluble sources of nutrients, as has been observed in other studies (Sousa et al., 2021; Almeida et al., 2022). However, these

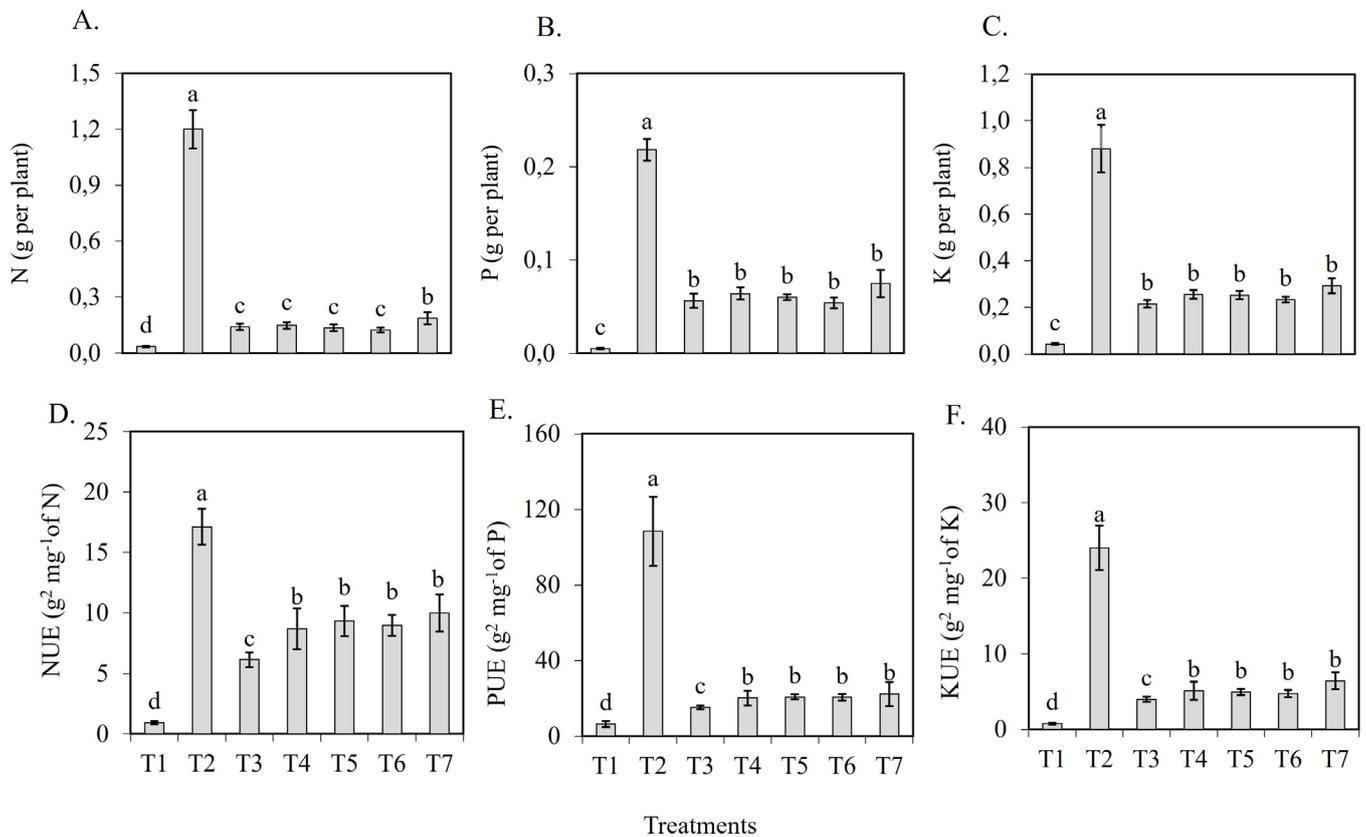
materials can improve the performance of plants compared to non-fertilized plants, possibly by replacing part of the mineral fertilizer that would be provided by soluble sources (Curtis et al., 2023).

The accumulated amounts of nitrogen (N), phosphorus (P), and potassium (K) in the maize dry mass, as well as the values of nutrient use efficiency, were higher in the T2 treatment (Figure 4). As with the variables related to plant growth, treatments T3, T4, T5, T6, and T7 promoted higher values of accumulation (Figures 4A, B, and C) and nutrient use efficiency (Figures 4D, E, and F) compared to treatment T1 (control), with emphasis on treatment T7 for the N accumulation variable and treatments T4, T5, T6, and T7 for nutrient use efficiency. Compared to the control treatment, the addition of cattle manure to the mining residue (T4) increased the nutrient utilization efficiency values to the same level as the treatments with the addition of *B. subtilis* and/or *B. amyloliquefaciens* (T5, T6, and T7).



T1- Soil without fertilization; T2 - Soil fertilized with soluble sources; T3 - Application of vermiculite residue (VR); T4 - VR enriched with cattle manure (CM); T5 - VR+CM with *Bacillus subtilis*; T6 - VR+CM+ *B. amyloliquefaciens*, and T7 - VR+CM+ *B. subtilis* + *B. amyloliquefaciens*). Data are means ± S.E. Lowercase letters compare the treatments by the Scott-Knott test at 5% significance level

**Figure 3.** Plant height (A), culm diameter (B), leaf dry mass - LDM (C), culm dry mass - CDM (D), and total dry mass -TDM (E) of maize plants as a function of the treatments



T1- Soil without fertilization; T2 - Soil fertilized with soluble sources; T3 - Application of vermiculite residue (VR); T4 - VR enriched with cattle manure (CM); T5 - VR+CM with *Bacillus subtilis*; T6 - VR+CM+ *B. amyloliquefaciens*, and T7 - VR+CM+ *B. subtilis* + *B. amyloliquefaciens*). Data are means ± S.E. Lowercase letters compare the treatments by the Scott-Knott test at 5% significance level

**Figure 4.** Accumulation of nitrogen-N (A), phosphorus-P (B), potassium-K (C), use efficiency of nitrogen-NUE (D), phosphorus-PUE (E), and potassium-KUE (F) of maize plants as a function of the treatments

The accumulation of nutrients is a consequence of dry mass production and the ability of the plant to absorb nutrients from the soil solution (Marschner, 2012). The application of soluble nutrients (T2) to the soil with low levels of P and organic matter promoted N, P, and K accumulations eight times (N) and 3.5 times (P and K) higher than the average of treatments T3, T4, T5, T6, and T7. However, the superiority of these treatments compared to the control indicates that the vermiculite residue should not be neglected, pointing to a potential use as a supplementary source of nutrients or soil conditioner (Silva et al., 2021; Marcelino et al., 2022).

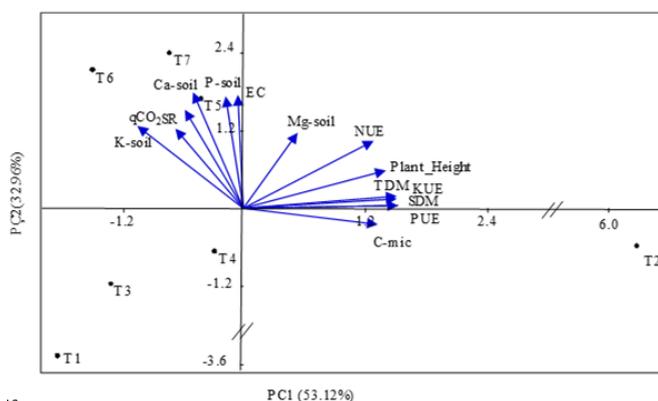
The factor analysis resulted in two factors (F). Factor 1 (PC1) has the most significant contribution power, with 53.12% of data variability, and is related to the growth and nutrient accumulation in maize, such as SDM, TDM, NUE, PUE, KUE, and plant height. Factor 2 (PC2) contributed with 32.96% of the data variability and relates soil data, such as EC, P-soil, Ca-soil, and Soil Resp. The sum of the variance of the two factors corresponds to 85.98% (Table 4).

In Principal Component Analysis (PCA), the vector projection diagram and the distribution of the point cloud in PC1 indicate that the variables SDM, TDM, NUE, PUE, KUE, and plant height had greater weight for T2 (soil fertilized with soluble sources) (Figure 5). Fertilization with soluble nutrients increases the growth and N-P-K utilization efficiency. However, in PC2 the variables EC, P-soil, Ca-soil, K-soil, Mg-soil, Soil Resp (SR),  $qCO_2$ , and NUE have greater weight for treatments T5 (VR+CM with *Bacillus subtilis*), T6 (VR+CM+ *B. amyloliquefaciens*), and T7 (VR+CM+ *B. subtilis* + *B. amyloliquefaciens*) (Figure 5). Treatments T5, T6, and T7 increase soil fertility, leaf dry mass, and nitrogen use efficiency. Bioinoculants benefit soil fertility, increasing nutrient and organic matter content and soil sustainability for new crops.

**Table 4.** Eigenvalues, percentage of total variance explained, in the multivariate analysis of variance (MANOVA) and correlations (r) between original variables and the principal components

Parameter	Principal components	
	PC1	PC2
Eigenvalues ( $\lambda$ )	7.97	4.94
Percentage total variance (S2%)	53.125	32.96
Hotelling test for treatments (T)	0.01	0.01
Variables	Correlation coefficient	
EC	-0.01431	0.4056
P-soil	-0.04189	0.4004
Ca-soil	-0.1181	0.4155
Mg-soil	0.1216	0.2725
K-soil	-0.2454	0.2957
SDM	0.3507	0.009216
TDM	0.3499	0.04616
NUE	0.3023	0.2261
PUE	0.3508	0.01328
KUE	0.3501	0.03993
Plant height	0.3222	0.1378
Soil Resp (SR)	-0.1381	0.352
C-mic	0.3048	-0.05708
$qCO_2$	-0.1554	0.2859

EC - Electrical conductivity; SDM - Culm dry mass; TDM - Total dry mass; NUE - Nitrogen use efficiency; PUE - Phosphorus use efficiency; KUE - Potassium use efficiency; C-mic - Microbial biomass carbon, and  $qCO_2$  - Metabolic quotient



**Figure 5.** Two-dimensional projection of the score variables analyzed in the two principal components (PC1 and PC2) for bio-inoculation of vermiculite residue, associated or not with cattle manure, on the release of nutrients and initial growth of maize

In the present study, it was demonstrated that vermiculite mining residue can contribute to improving soil fertility, especially in soils with low calcium and potassium contents. However, longer studies, conducted until the production phase, using crops with high nutrient extraction capacity, can provide more accurate information about the potential use of this residue, whether or not it is enriched with cattle manure and/or bacteria capable of solubilizing silicate minerals.

## CONCLUSIONS

1. Regardless of the use of cattle manure or beneficial microorganisms, vermiculite mining waste increased the availability of Ca and K in the soil, but was not able to match the soluble sources of nutrients.
2. Mining waste associated with cattle manure and beneficial microorganisms (*Bacillus subtilis* and *B. amyloliquefaciens*) increased microbial activity and enhanced the release of available calcium for maize.
3. Vermiculite mining waste increased the growth and the dry mass production of maize compared to the non-fertilized soil, but was consistently inferior to conventional fertilization.

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