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Olivine melilitite powder applied in association with bacterial inoculation impacts soil microbiological attributes

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ABSTRACT: This study aimed to evaluate the impact of the combination of olivine melilitite powder with bacterial inoculation on soil microbiological attributes. The experiment was conducted in a randomized block design (n = 4), with the following treatments: chemical fertilizer (CF), fertilizer reduction + remineralizer (FRR), fertilizer reduction + remineralizer + *Azospirillum* (FRA), fertilizer reduction + remineralizer + *Bacillus* (FRB), and fertilizer reduction + remineralizer + co-inoculation of *Azospirillum* and *Bacillus* (FRC). A 25 % reduction of chemical fertilizer was tested in FRR, FRA, FRB, and FRC for two years. The microbial biomass carbon (MBC), soil basal respiration (SBR), microbial quotient (qMIC), metabolic quotient (qCO_2), and grain yield (GY) of maize were evaluated. The results indicated that the soil from treatments FRA (515.72 mg microbial C (carbon) and 2.28 %), FRB (547.43 mg microbial C and 2.65 %), and FRC (529.64 mg microbial C and 2.38 %) exhibited the greater values of MBC and qMIC. The highest qCO_2 values were found in the soil of FRR (5.55 μg C-CO_2 μg MBC) and CF (5.84 μg C-CO_2 μg MBC), indicating stress effects on the microbial community. However, following the first cultivation, this effect was reduced in FRR due to the application of olivine melilitite. Moreover, MBC (R^2 = 0.46), qMIC (R^2 = 0.35), and SBR (R^2 = 0.79) exhibited a positive correlation with maize GY, while qCO_2 (R^2 = –0.33) presented a negative correlation with GY. This suggests that bacterial inoculation associated with olivine melilitite may have influenced the results. In conclusion, the application of olivine melilitite and inoculation with beneficial bacteria has been demonstrated to enhance soil microbiological attributes and maize yield.

Keywords: *Bacillus megaterium*, *Bacillus subtilis*, *Azospirillum brasilense*, soil remineralizers, rock powder

Introduction

Remineralizers are agricultural inputs of mineral origin that can to alter the physical-chemical properties of soils (Ramos et al., 2022). Olivine melilitite is a silicate of igneous origin with a favorable chemical and mineralogical composition for agricultural use (Mazzeo et al., 2021). Olivine melilitite powder has been demonstrated to affect the chemical properties of soils, including nutrient content, cation exchange capacity, hydrogen potential, potential acidity, and the sum of bases (Almeida et al., 2022). However, its impact on soil microbiota remains to be fully elucidated.

Historically, low solubility has been identified as a significant limitation in the agricultural use of remineralizers (Luchese et al., 2021). The bacteria *Bacillus subtilis* (C.), *Bacillus megaterium* (B.) (Baptista et al., 2022), and *Azospirillum brasilense* (T.) (Ribeiro et al., 2018) have been the subject of study due to their capacity to solubilize minerals through the synthesis of organic acids, extracellular substances and surface solubilization (Aloo et al., 2024). Consequently, the utilization of solubilizing bacteria that promote plant growth represents a potential avenue for accelerating the solubilization impact of olivine powder and enhancing the microbiological properties of the soil.

Research on remineralizers seeks to evaluate the agronomic effects on grain-producing crops, primarily focusing on the physical-chemical attributes of soils (Luchese et al., 2023). However, the functional microbiota is not considered in these studies. It is important to note that microbiological indicators, such as microbial biomass carbon (MBC), soil basal respiration (SBR), microbial quotient (qMIC), and metabolic quotient (qCO_2), are highly sensitive to management changes in agroecosystems (Sakin et al., 2024). Therefore, these attributes can reveal changes related to the early application of olivine melilitite powder on the soil microbiota.

Therefore, it is hypothesized that applying olivine melilitite powder influences on the soil microbial attributes. Furthermore, the inoculation of specific microorganisms with the rock powder appears to accelerate the observed effects, thereby enhancing the biological quality of the soil and crop yield. The objective of this study was to evaluate the effect of the association of olivine melilitite powder with the bacterial strains *B. subtilis*, *B. megaterium*, and *A. brasilense* and the response of this management practice on the soil microbiological attributes.

Materials and Methods

Samples of olivine melilitite were subjected to mineralogical characterization using X-ray diffractometry

(XRD) and chemical characterization using chemical methods with quantification of the elements by induction-coupled plasma (ICP). The results of these analyses are presented in Table 1.

Studies employing remineralizers of varying compositions and granulometries have tested doses ranging from 1 to 100 t ha^{-1} (Swoboda et al., 2022). However, the most commonly utilized dose for field experiments oscillates between 2.5 and 15 t ha^{-1} (Almeida-Júnior et al., 2020; Soratto et al., 2021; Luchese et al., 2023). The use of olivine melilitite powder with a particle size smaller than 0.35 mm was in accordance with normative instruction N° 05 of 2016 (MAPA, 2016) for the *Filler* category. The application of olivine melilitite powder was divided as follows: 2.5 t ha^{-1} in top dressing and 2.5 t ha^{-1} incorporated into row placement along with the chemical fertilizer, resulting in a total dose of 5 t ha^{-1} in the treatment plots that received this input.

The evaluation period encompassed two years, during which successive maize *Zea mays* (L.) crops were cultivated during the 2019/20 and 2020/21 harvests. The study was initiated at the time of the application of the remineralizers in an area specifically managed for the study. The cultivation system employed was the unconsolidated direct planting, implanted from the installation of the experiment. The efficacy of a reduced dose of soluble fertilizer recommended for maize was examined during the two-year- evaluation period. This involved the use of olivine melilitite powder as a source of nutrients and the examination of the potential of bacteria *B. subtilis*, *B. megaterium*, and *A. brasilense* in optimizing agronomic effects resulting from the reduction of soluble fertilizer nitrogen, phosphorus, and potassium (NPK).

Table 1 – Chemical and mineralogical composition of olivine melilitite.

Mineral composition			
		%	
Principal	Melilite	40.0	
	Phlogopite	30.0	
	Clropyroxene	15.0	
	Olivine	10.0	
	Opaque minerals	5.0	
Alteration minerals	Iron Oxides	Trace	
	Clay minerals	Trace	
	Talc	Trace	
Chemical composition			
Chemical elements		Oxides	
	%		%
Silicon (Si)	22.7	SiO_2	37.5
Magnesium (Mg^{+2})	10.4	MgO	17.4
Calcium (Ca^{+2})	10.6	CaO	14.8
Iron (Fe^{+2})	7.3	Fe_2O_3	10.5
Potassium (K^{+})	2.3	K_2O	2.7
Phosphorus (P)	0.5	P_2O_5	1.2
	mg kg^{-1}		
Sulfur (S)	3,300		
Manganese (Mn)	1,500		
Zinc (Zn)	94.0		
Copper (Cu)	63.0		
Cobalt (Co)	57.0		
Nickel (Ni)	385.0		

The initial crop season commenced in Dec 2019, followed by the second one in Oct 2020. The objective was to test a 25 % reduction in the recommended chemical fertilization in a randomized block design (n = 4) using the following treatments: chemical fertilizer (CF), fertilizer reduction + remineralizer (FRR), fertilizer reduction + remineralizer + *Azospirillum* (FRA), fertilizer reduction + remineralizer + *Bacillus* (FRB), and fertilizer reduction + remineralizer + co-inoculation of *Azospirillum* and *Bacillus* (FRC). The aim was to investigate the impact of a 25 % reduction of chemical fertilizer on crop yield and quality. It should be noted that the co-inoculation technique consisted of the simultaneous application of multiple microbial groups, in this case, represented by the inoculation of bacterial *A. brasilense*, *B. subtilis*, and *B. megaterium*. The maize hybrid utilized was FS 2B512 PW, planted in a 45 cm spacing between rows and a population of 60,000 plants ha^{-1}.

The calculations for selecting the formula and dose of the chemical fertilizer for the 2019/20 crop season were based on the interpretation of the nutrient contents from the area before planting and for the 2020/21 crop season. The soil sampling was conducted in the predecessor crop (Table 2), in accordance with the Liming and Fertilization Manual for the states of Rio Grande do Sul and Santa Catarina, Brazil (SBCS, 2016).

Subsequently, the samples were subjected to chemical analysis to determine the levels of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity (CEC), and hydrogen potential (pH). The P content was quantified using a visible light spectrophotometer following extraction with Mehlich solution (hydrochloric acid 0.05 mol L^{-1} and sulfuric acid 0.0125 mol L^{-1}). The K content was determined by flame photometry after extraction with Mehlich solution. The Ca and Mg contents were determined in an atomic absorption spectrophotometer using an extraction in potassium chloride solution (1 mol L^{-1}). The sum of the cations Ca^{+2}, Mg^{+2}, K^{+}, and potential acidity calculated the CEC. The pH was determined with a potentiometer in a 1:1 soil-water ratio.

Inoculation and co-inoculation were performed in a row placement with the bacterial strains ABV5 and ABV6 of *A. brasilense*; BRM 2084 from *B. subtilis* and BRM 119 from *B. megaterium*. Commercial inoculants were utilized with 4 × 10^{9} colony-forming units per application and a concentration equivalent to 120 mL ha^{-1}.

Table 2 – The contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), total organic carbon (TOC), and the values of cation exchange capacity (CEC), hydrogen potential (pH) in the pre-cultivation period for the 2019/20 and 2020/21 crop years.

Treatments	P	K	Ca	Mg	TOC	CEC	pH
	------------------ mg dm^{-3} ------------------		---------------- cmol$_c$ dm^{-3} ----------------		g kg^{-1} soil	cmolc dm^{-3}	-
				Pre-cultivation period			
TA	13.30	120.00	2.80	1.90	2.03	10.30	4.90
				2019/20 crop year			
CF	16.58	210.55	2.71	2.53	2.18	10.08	5.55
FRR	18.05	237.94	3.18	2.53	2.23	10.05	5.82
FRC	41.51	315.57	3.15	2.35	2.22	10.23	5.68
FRA	37.98	327.75	3.13	3.03	2.26	10.64	5.93
FRB	35.03	312.55	4.15	2.90	2.06	11.77	5.83
				2020/21 crop year			
CF	14.15	263.25	3.20	3.53	1.91	16.84	5.23
FRR	15.81	230.00	3.48	3.70	1.97	15.51	5.25
FRC	14.10	266.00	6.65	4.07	2.07	16.06	5.68
FRA	15.58	277.00	6.35	3.78	2.00	16.10	5.35
FRB	21.98	288.00	6.90	4.23	2.01	15.91	5.48

TA = total area; CF = chemical fertilizer; FRR = fertilizer reduction + remineralizer; FRA = fertilizer reduction + remineralizer + *Azospirillum*; FRB = fertilizer reduction + remineralizer + *Bacillus*; FRC = fertilizer reduction + remineralizer + co-inoculation.

Soil samples were collected at the end of the cultivation cycles in nine randomly selected points within the plots from the 0-10 cm layer. The samples were homogenized to create a composite sample and sieved in a 2-mm mesh to analyze the variables. These included the microbial activity measured by soil basal respiration (SBR), the calculation of the metabolic quotient (qCO_2), microbial biomass carbon (MBC), microbial quotient (qMIC), and total organic carbon (TOC). Additionally, grain yield (GY) was estimated at the end of each maize crop cycle.

The fumigation-extraction method (Vance et al., 1987) determined the microbial biomass carbon (MBC) with three fumigated and three non-fumigated analytical replicates. The fumigation was carried out in a desiccator, from the incubation of the samples with ethanol-free chloroform ($CHCl_3$) for 24 h at 26° C in the absence of light. Aliquots were extracted by stirring in a 0.5 mol L^{-1} potassium sulfate (K_2SO_4) extracting solution, followed by decantation, filtration, and oxidation with 66.7 mmol L^{-1} potassium dichromate ($K_2Cr_2O_7$) in a boiling tank at a temperature of 90 °C for 1 h. The soluble carbon (C) content was determined by titration in an ammoniacal ferrous sulfate solution ($Fe(NH_4)_2(SO_4)_2(H_2O)_6$) with a concentration of 33.3 mmol L^{-1} in the presence of a diphenylamine indicator (1 %), with the difference between the carbon extracted from fumigated and non-fumigated soil calculated.

The microbial activity was evaluated by determining the SBR (mg C – CO_2 kg^{-1} soil h^{-1}) from 50 g of soil incubated at a temperature of 28° C until the CO_2 emission stabilized, a period that varied from 15 to 18 days. The released CO_2 was captured in a 0.5 mol L^{-1} sodium hydroxide (NaOH) solution, precipitated with a 0.5 mol L^{-1} barium chloride solution ($(BaCl_2)_2(H_2O)$) and quantified by titration of the remaining NaOH in 0.1 mol L^{-1} hydrochloric acid (HCl) solution in the presence of phenolphthalein indicator (1 %) (Alef and Nannipieri, 1995). The SBR and MBC results were employed to calculate the qCO_2. The TOC (g kg^{-1}) was determined by near-infrared spectroscopy (NIRS), and the microbial quotient (qMIC) was calculated from the MBC results. The GY was determined from manual harvesting in triplicate of 1 m^2 in the useful area of each plot and drying to correct the humidity at 13 % to calculate the yield (kg ha^{-1}).

The microbiological data were subjected to the analysis of variance and, when significant, the means were grouped using the Scott-Knott test at 5 % probability ($p < 0.05$). The grain yield was then compared with the control by the Dunnett's test at 5 % probability ($p < 0.05$). The regression analyses were performed using multiple linear models to explain the correlation between microbiological attributes (independent explanatory variables) and grain yield (dependent variable) at 5 % probability ($p < 0.05$). The multivariate exploration was carried out by initially identifying the multicollinearity of the variables tested using the Variance Inflation Factor and applying Forward Selection to identify significant variables, followed by the principal component analysis (PCA) to verify the spatial distribution of treatments along the variables.

Results

The analysis of variance revealed a difference between the treatments tested for the variables MBC, qMIC, qCO_2, and SBR over the two years of cultivation. The highest MBC and qMIC values were found in the soil of the treatments that received the association of the remineralizer with the bacteria (FRA, FRB, and FRC). Additionally, there was a difference between the CF and FRR in the 2020/21 crop (Table 3).

The qCO_2 values were found to be higher in the CF and FRR treatments in 2019/20, with the lowest averages observed in the treatments that employed the application of olivine melilitite powder in conjunction with inoculation (FRA and FRB) or co-inoculation (FRC) as a result of the reduction in chemical fertilizers (Table 3). In the 2020/21 crop year, the highest values of SBR and qCO_2 were observed in the soil of the CF treatment, indicating a difference in comparison to the FRR treatment. Conversely, the lowest metabolic quotients were observed in the soils of the treatments that applied olivine melilitite powder in conjunction with bacteria *B. subtilis*, *B. megaterium*, and *A. brasilense* through inoculation (FRA and FRB) or co-inoculation (FRC).

In the 2019/20 crop season, GY in FRR exhibited a difference with approximately 2,000 kg ha^{-1} below the yield observed for the control treatment (CF) (Figure 1A). This effect was maintained in the 2020/21 crop, where FRR yield was approximately 900 kg ha^{-1} below that observed in the control. Conversely, the FRB treatment showed an increase of approximately 700 kg ha^{-1} in comparison to the control (Figure 1B).

It was observed that the MBC, qMIC, SBR, and qCO_2 were related to the maize yield in the two evaluated crops, showing that these indicators impacted GY in the tested conditions. The multiple linear regression model was statistically significant (p < 0.001) with an adjusted coefficient of determination R^2 = 0.71 (Figure 2). When

Table 3 – Indicators of microbial biomass carbon (MBC), microbial quotient (qMIC), soil basal respiration (SBR), metabolic quotient (qCO_2) and total organic carbon (TOC) in the 2019/20 and 2020/21 crop years.

Treatments	MBC	qMIC	SBR	qCO_2	TOC
	mg microbial C kg^{-1} soil	%	mg C-CO_2 kg^{-1} soil	µg C-CO_2 µg MBC	g kg^{-1} soil
			2019/20 crop year		
CF	344.58 ± 31.63 b*	1.58 ± 0.16 b*	1.23 ± 0.20^{ns}	5.84 ± 0.31 a*	2.17 ± 0.08^{ns}
FRR	368.35 ± 44.41 b	1.65 ± 0.13 b	1.30 ± 0.11	5.55 ± 0.44 a	2.23 ± 0.12
FRC	529.64 ± 38.09 a	2.38 ± 0.18 a	1.24 ± 0.17	3.45 ± 0.35 b	2.21 ± 0.20
FRA	515.72 ± 27.33 a	2.28 ± 0.09 a	1.25 ± 0.19	3.46 ± 0.27 b	2.26 ± 0.17
FRB	547.43 ± 33.13 a	2.65 ± 0.11 a	1.31 ± 0.12	3.44 ± 0.33 b	2.11 ± 0.24
p-value	0.001	0.001	0.809	0.001	0.912
F-value	15.69	13.12	0.394	84.88	0.564
			2020/21 crop year		
CF	417.49 ± 45.30 c*	1.97 ± 0.15 c*	0.89 ± 0.10 a*	6.67 ± 0.58 a*	2.12 ± 0.18^{ns}
FRR	552.66 ± 39.77 b	2.70 ± 0.08 b	0.55 ± 0.12 b	3.66 ± 0.30 b	2.04 ± 0.11
FRC	728.19 ± 40.03 a	3.40 ± 0.25 a	0.54 ± 0.17 b	2.42 ± 0.37 c	2.14 ± 0.20
FRA	675.36 ± 35.94 a	3.31 ± 0.21 a	0.56 ± 0.19 b	2.87 ± 0.10 c	2.05 ± 0.23
FRB	755.51 ± 51.83 a	3.65 ± 0.31 a	0.52 ± 0.22 b	2.72 ± 0.15 c	2.08 ± 0.09
p-value	0.001	0.001	0.001	0.001	0.961
F-value	21.16	18.01	35.51	166.98	0.365

CF = chemical fertilizer; FRR = fertilizer reduction + remineralizer; FRA = fertilizer reduction + remineralizer + *Azospirillum*; FRB = fertilizer reduction + remineralizer + *Bacillus*; FRC = fertilizer reduction + remineralizer + co-inoculation. *Means followed by the same letters in the columns do not differ statistically by the Scott-Knott test (p < 0.05). ^{ns}Analysis of variance not significant.

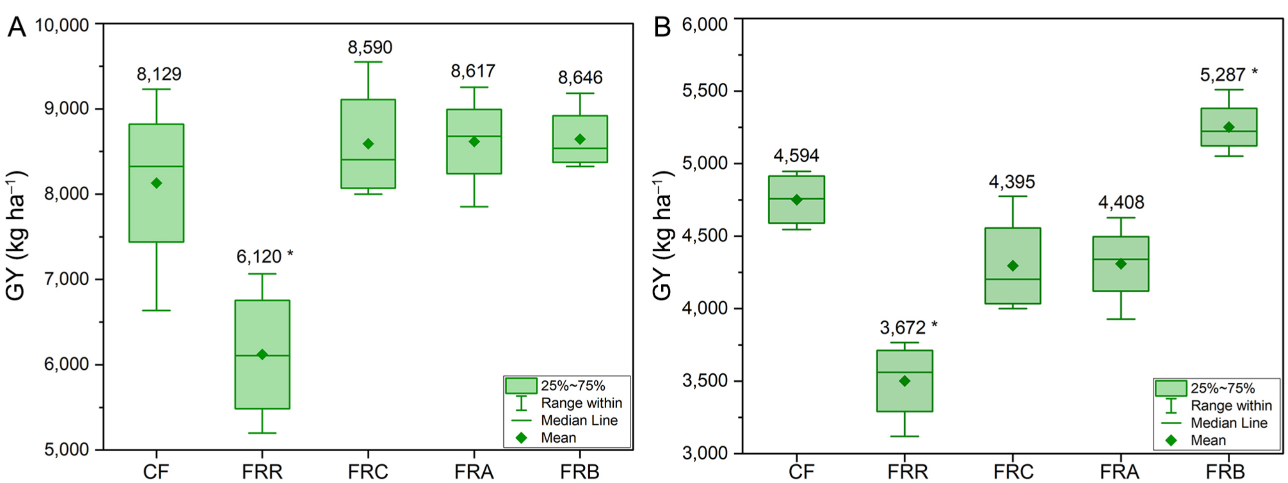

Figure 1 – A) Grain yield (GY) of 2019/20 maize crop; B) Grain yield (GY) of 2020/21 maize crop, of treatments: CF = chemical fertilizer; FRR = fertilizer reduction + remineralizer; FRA = fertilizer reduction + remineralizer + *Azospirillum*; FRB = fertilizer reduction + remineralizer + *Bacillus*; FRC = fertilizer reduction + remineralizer + co-inoculation. *Different from control by Dunnett's test (p < 0.05).

considered individually, the variables MBC (R^2 = 0.46), qMIC (R^2 = 0.35), and SBR (R^2 = 0.79), represented by the blue color, demonstrated a positive correlation with maize yield. In contrast, the qCO_2, represented by the red color, exhibited a negative effect when correlated with GY (R^2 = –0.33).

The principal component analysis (PCA) illustrates the spatial distribution of the data in conjunction with the treatments across the two crop seasons under evaluation. In the PCA for the 2019/20 crop season, the principal component (PC) 1 explained 61 %, while PC 2 explained 26 %, representing 87 % of data variability, being MBC, qMIC and qCO_2 the variables that most to both dimensions (Figure 3A). It can be observed that GY is associated with FRA, FRB and FRC treatments, which indicates a relationship with MBC and qMIC. Conversely, qCO_2 is associated with CF and FRR treatments, which exhibited a distinct relationship from GY.

The spatial dispersion of data from the 2020/21 crop, represented by the PCA in Figure 3B, indicates that PC 1 explained 64 % while PC 2 explained 24 %, accounting for 88 % of the data variability. As observed in the previous crop season, the FRA, FRB, and FRC treatments are associated with the MBC and the qMIC, yet these variables are not linked to crop yield in this ordering model. The displacement of the FRR to an intermediate range of dispersion is noteworthy, as it is dissociated from all the variables, when at first it was associated with high rates of basal respiration and CO_2 emission, with the CF being associated with the variables SBR and qCO_2.

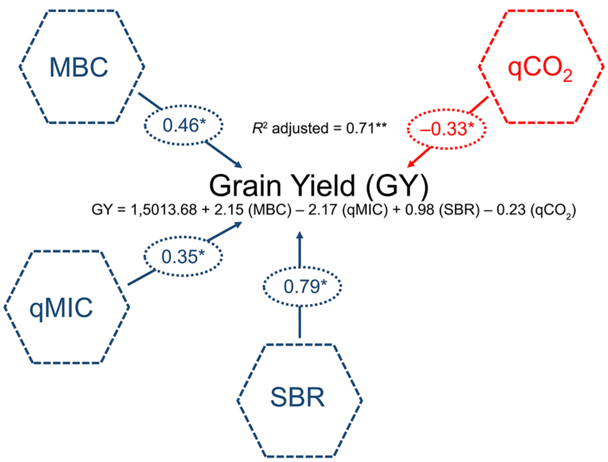

Figure 2 – Representative of the multiple linear regression model with the dependent variable grain yield (GY) and independent microbial biomass carbon (MBC), microbial quotient (qMIC), metabolic quotient (qCO_2) and soil basal respiration (SBR). *95 % confidence level (p < 0.05). **99 % confidence level (p < 0.001)

Discussion

Microorganisms, through inoculation, promotes soil conditioning, modify interactions in the rhizosphere, and positively affect microbial biomass in shorter periods when compared to changes in soil organic matter (Korenblum et al., 2020). Therefore, given that there was no difference in TOC for the evaluated treatments, a difference that was already anticipated due to the time since the implementation of the no-tillage system (NT), it is postulated that the increase in MBC in the soil of the FRA, FRB, and FRC treatments is the result of inoculation with bacterial strains *B. subtilis*,

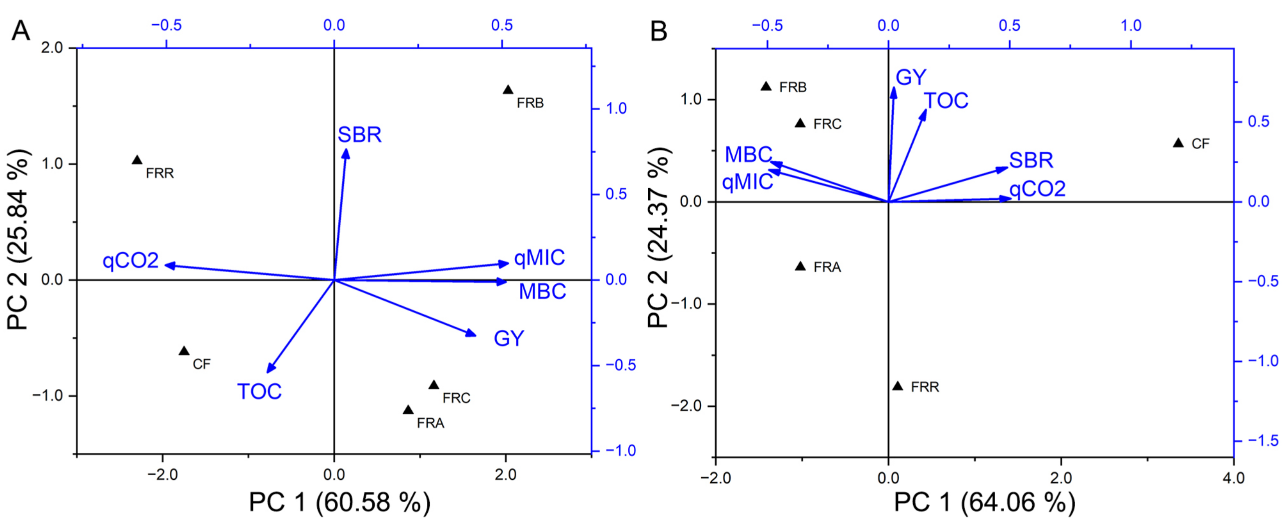

Figure 3 – A) Principal component analysis (PCA) of the 2019/2020 maize crop; B) Principal component analysis (PCA) of the 2020/2021 maize crop, using grain yield (GY) variables, microbial biomass carbon (MBC), microbial quotient (qMIC), metabolic quotient (qCO_2), total organic carbon (TOC) and soil basal respiration (SBR) of treatments: CF = chemical fertilizer; FRR = fertilizer reduction + remineralizer; FRA = fertilizer reduction + remineralizer + *Azospirillum*; FRB = fertilizer reduction + remineralizer + *Bacillus*; FRC = fertilizer reduction + remineralizer + co-inoculation.

B. megaterium, and *A. brasilense* in combination with the synergism of microbial conditioning promoted by olivine melilitite powder.

The distinction between CF and FRR in the 2020/21 season demonstrates that the application of olivine powder can positively influence the MBC and qMIC of the soil, even in the absence of inoculation. However, the qMIC values indicated a greater incorporation of microbial C in the soil of the FRA, FRB, and FRC treatments. These attributes reflect the reserve of immobilized C by the soil microbiota (Balota et al., 2014; Pompeo et al., 2017). It is therefore postulated that the bacteria stimulated microbial growth, with a greater contribution of microbial biomass to microbial C in the soil of the treatments that received the association of the remineralizer and the inoculation.

Given that the biomass consists of microbial cells (fungi, bacteria, and actinomycetes) and represents the living portion of the soil (Stockdale and Brookes, 2006), the increase in MBC and qMIC may be indicative of the entry of microorganisms through inoculation and the stimulation of greater multiplication, cell growth or activation of existing communities in the soil as a result of the application of olivine melilitite powder. Remineralizers can restore soil life by adding minerals that serve as an energy source for microbial communities (Hamaker and Weaver, 2002). Therefore, the significant reduction in the qCO_2 of the FRA, FRB, and FRC treatments indicates that the association of bacteria with the olivine powder provided more stable conditions, improving the efficiency of the microbial community of these soils in the use of C through the conversion into microbial biomass.

Because of this scenario, the hypothesis that incorporating olivine powder affects soil microbiological attributes can be validated. The verified microbiological data, as observed through the FRR, FRA, FRB, and FRC treatments, indicate that the incorporation of C was significantly enhanced in the presence of olivine melilitite, particularly in the second year following its application. This finding corroborates the hypothesis that the effect of rock dust is a gradual process that can be optimized by bacterial action through inoculation. It should be noted that the association of bacteria through inoculation appears to stimulate microbial growth and C cycling in the agroecosystem during the first year of application, resulting in a higher microbial quality of the soil compared to the use of soluble fertilizers as the sole source of nutrients.

The high emission of CO_2 by soil microorganisms may indicate an inefficient microbial community (Antisari et al., 2021) or represent a reflection of the high activity of microorganisms due to the greater amount of available energy (Okolo et al., 2020). High values of qCO_2 indicate that the microbial community may be oxidizing cellular C to maintain its metabolism (Giagnoni and Renella, 2022) since, under stress conditions, microorganisms require a greater amount of energy for their maintenance (Araujo et al., 2019).

In this context, the elevated qCO_2 level observed in the CF and FRR in the 2019/20 crop year suggests that the energy expenditure is greater in the soil of these treatments for the maintenance of microbial metabolism, due to the lower capacity to convert C into microbial biomass. In addition, the isolation of CF in the SBR and the resulting qCO_2 in 2020/21 directly reflect the reduction in MBC and qMIC observed in the 2020/2021 crop year. Given that microorganisms metabolize substances to biosolubilize minerals and extract elements of interest (Dong, 2010; Uroz et al., 2015), it can be posited the soil microbiota may be benefiting from the solubilization of olivine melilitite powder. Furthermore, the application of the total dose of chemical fertilizer increased soil microbial activity, indicating a stress situation due to the high qCO_2 and lower MBC verified in the CF soil.

In general, chemical fertilizers have a high concentration of highly soluble inorganic ions in their composition, which is why the salt content of these inputs is high (Gulick et al., 2023). In contrast, remineralizers are less aggressive natural sources, with a lower concentration and solubilization of nutrients (Theodoro et al., 2021). It is, therefore, assumed that applying chemical fertilizers as the sole source of nutrients results in greater microbial respiration, which reduces the efficiency of C incorporation by microbial biomass. In this context, the use of a remineralizer in conjunction with bio-inputs minimizes the stress experienced by the soil biota.

In the 2020/21 crop year, the use of remineralizer, even without inoculation and with a reduction of the chemical source of nutrients, promoted improvements in the metabolic efficiency of soil organisms compared to applying soluble fertilizer as the sole source of nutrients. Conversely, degradation of organic matter by microorganisms requires a greater expenditure of energy (Bahl et al., 2021). Therefore, the access of microorganisms to nutrients contained in the mineral structure can increase their metabolic rate and result in greater energy expenditure in a shorter period of time. This phenomenon can explain the effect observed in the soil of FRR in the 2019/20 crop year, where the application of olivine melilitite increasedqCO_2. However, to substantiate these findings, further investigation is required, focusing on the isolated action of bacteria in the soil and their interaction with soluble fertilizers for extended periods.

The data indicate that the soluble chemical source of nutrients negatively affected microbial metabolism when applied in full dose. Furthermore, the application of the remineralizer modified the behavior of the microbial community, improving the biological quality of the soil, mainly when associated with inoculants. In the 2019/20 crop season, applying the remineralizer with a reduction in soluble fertilizer without inoculation techniques was not a viable alternative. This result validated the hypothesis that the effect of the remineralizer can be optimized through the inoculation of bacteria, which in turn promotes a better response in terms of crop yields through improvements in soil biological properties.

The use of the remineralizer showed promising results. Over the two-year-experimental period, there was an observable evolution in the microbiological indicators of soil quality in relation to the use of this input. Historically, the use of remineralizers has been directed toward alternative agricultural models (Gomes et al., 2014; Swoboda et al., 2022), where the biological conditions of the soil favor the effects of this input due to microbial action. However, agricultural systems are susceptible to disruption and naturally present less biological activity due to anthropogenic influences (Baiamonte et al., 2015). Consequently, the efficacy of remineralizers is contingent upon the implementation of management practices in agricultural areas and the maintenance of optimal biological conditions in the soil.

Microbial mechanisms are presented as essential tools in the release (Aloo et al., 2024) and conversion of nutrients (Berde et al., 2021). Therefore, inoculation techniques and studies with different microbial strains can optimize the action of olivine melilitite powder under the management and soil conditions tested. The bacterial inoculation may have affected the microbial quality, favoring the action of olivine melilitite on the biological indicators of the soil. Accordingly, the outcomes observed over the two-year-experimental period should be interpreted as a synergistic effect of the management strategies proposed in this study. To substantiate this finding, it is essential to conduct a separate evaluation of the bacteria strains involved.

A significant result of this research is the correlation identified between the variables through the multiple linear regression models. Linear determination coefficients demonstrate a relationship between MBC, qMIC, and SBR and grain yield. This effect may be associated with enhanced efficiency of soil microbiota or stimuli provided by inoculation. Studies have demonstrated that inoculation with bacteria *B. subtilis* and *B. megaterium* can result in gains of 516 to 1,182 kg ha^{-1} in maize yield (Sousa et al., 2021). Therefore, it can be concluded that an increase in yield after inoculation is a real phenomenon. However, an increase in qCO_2 caused by high basal respiration rates and lower incorporation of C by the microbial community has been observed to reduce grain yield.

The biological indicators MBC and qMIC are broadly related to management practices (Bedolla-Rivera, et al., 2020), representing attributes closely linked to soil fertility (Silva-Aragão et al., 2020). It is important to note that soil biology can facilitate plants' search for nutrients. This process improves the conditions for the functioning of absorption mechanisms for elements, regardless of the levels available in the soil (Bagyaraj et al., 2016). In this context, the crop yield can be high as a reflection of the biological conditions and not necessarily of the increase in the levels of nutrients in the soil. This is because the solubilization of the remineralizer is slow, and the applied dose is insufficient to raise the levels of nutrients in the face of the 25 % reduction of soluble fertilizer.

The application of rock dust without bacterial inoculation (FRR treatment) showed the lowest yields in relation to the control in both years of evaluation, indicating that the utilization of olivine melilitite powder without inoculation constrained maize productivity. The remineralizers present a lower concentration and slower solubilization of nutrients in their composition (Theodoro et al., 2021). Consequently, the FRR treatment exhibited lower yield responses thana the control. Nevertheless, in the 2020/21 crop season, the application of the remineralizer in conjunction with inoculation with *B. subtilis* and *B. megaterium* resulted in a higher GY in comparison to the control. This management strategy proved to be more effective when attempting to utilize the olivine powder in a manner that reduces the use of soluble fertilizers.

Inoculation with *B. subtilis* has enhanced maize yield (Sousa et al., 2021). Therefore, it can be postulated that the impact on GY may be related to the action of bacteria. Bacteria *B. subtilis* (Velloso et al., 2020) and *A. brasilense* (Kazi et al., 2016; Santos et al., 2021) facilitate the solubilization of nutrients, improving the utilization of fertilizers and providing mineralized forms of soil nutrients (Kaur and Gosal, 2017). Furthermore, the action of bacterial communities in symbiosis with the roots promotes biological weathering (Soumare et al., 2023). The addition of olivine melilitite powder and inoculation promoted improvements of the biological activities in the soil, which may have supplemented the reduction in chemical fertilizer and enhanced maize grain yield. Therefore, the hypothesis is confirmed that the application of olivine powder and inoculation promote positive effects on maize grain yield.

The indicators SBR and qCO_2 play a dualistic role, as they can represent both a disturbance and the ecological balance (Xue et al., 2020). Therefore, a contextual analysis from an agronomic standpoint is necessary for a correct understanding. However, after the joint analysis of microbial attributes and GY, it is evident that high values of qCO_2 can negatively affect maize yield. The linear coefficients obtained in the multiple regression models demonstrate the influence of the microbiological indicators MBC, qMIC, SBR, and qCO_2 on the yield of the evaluated crop, taking into account the wide variability observed in these indicators.

The multivariate analysis is a tool to explain microbiological data (Kraft et al., 2021). Therefore, the PCA is important for identifying groups of treatments associated with the variables studied in spatial distribution plans. Regardless of the year of evaluation, the PCA indicates that the treatments that used the association of the remineralizer with the inoculation techniques promoted better results, influencing the yield of the evaluated crop.

Notably, the high rates of C-CO_2 found in FRR in the 2019/20 crop season indicate that the initial effects of the remineralizer application impact the soil microbial community, possibly due to changes in the soil energy matrix. Conversely, inoculation appears to provide more stable biotic conditions for the application of olivine melilitite powder, rendering the use of beneficial agents through inoculation with bacterial strains, such as *B. subtilis*, *B. megaterium*, and *A. brasilense*, a viable option to mitigate the initial effect of rock dust application. In addition, this approach can optimize the action of this input on the microbiological attributes of the soil.

After two years of cultivation, it was observed that the application of the remineralizer had an optimizing effect on the microbiological attributes, resulting in an increase in the activity of the soil microbiota and its efficiency in the incorporation of microbial C due to a reduction of CO_2 emissions. The increase in microbial C stocks reflects an improvement in soil quality (Almeida et al., 2021). Therefore, the application of olivine melilitite powder provided favorable conditions for the microbiota and improved soil quality.

The application of olivine melilitite powder in conjunction with inoculation with bacterial strains of *B. subtilis*, *B. megaterium*, and *A. brasilense* resulted in improvements in the microbiological attributes of the soil. The bacteria leveraged the effects of olivine melilitite powder on the microbial attributes of the soil, exhibiting pronounced effects from the first year of application. Furthermore, a clear correlation was observed between the microbial attributes and the productive response of the cultures. In the absence of inoculation, the remineralizer does not exhibit changes in microbial attributes during the first year of application due to its low solubility. However, significant changes are observed from the second year onward, reflecting a residual and cumulative effect.

A 25 % reduction in soluble chemical fertilization associated with the remineralizer and inoculation with organisms *B. subtilis*, *B. megaterium*, and *A. brasilense* allows for productive gains that are equal to or greater than those obtained in the use of complete soluble fertilization, promoting improvements in the microbiological quality of the soil. However, the study indicates the emergence of new hypotheses regarding the impact of the microbial response. Specifically, it raises the question of whether the positive response results from the remineralizer's association with the bacteria or is simply a consequence of the inoculation process itself. In light of these findings, it is recommended that the study be continued with evaluations conducted over extended periods and the introduction of new treatments to the experiment. This will facilitate the acquisition of new data that could be used to test the hypotheses raised, thereby expanding the knowledge base regarding the use of remineralizers.

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