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Soil Health Check-Up of Conservation Agriculture Farming Systems in Brazil

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Abstract: Conservation agriculture has been promoted as the main strategy to regenerate soil life but its effect on soil enzyme activity remains little documented. This study investigated the β-glucosidase and arylsulfatase enzymes as tools to evaluate soil health at the field level. Croplands in four main grain-producing states in Brazil were selected for this study. In each cropland, three environments (high yield (HYE), medium yield (MYE), and low yield (LYE)) were delineated for soil sampling to determine soil chemical attributes and enzyme activity. In one of these fields with a large temporal database, soil DNA characterization was also undertaken. The two soil enzymes investigated were affected by a range of soil attributes and the most important of these were identified. Around 40% of the data points sampled had low soil organic matter content; these were associated with low enzyme activity. Furthermore, in HYE there was more biodiversity and a higher presence of plant-growth promoters, while in LYE there were more plant pathogenic organisms.

Keywords: Brazil agro-ecoregions; soil biodiversity; soil enzymes; organic matter

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1. Introduction

The projected global population growth over the coming decades will increase the demand for food, fibre, biofuel, energy, water, and other agricultural products. In consequence, there will be growing pressure on natural ecosystems and agroecosystems, which are already facing sustainability challenges due to climate change and increasing soil degradation linked to loss of biodiversity, compromising a range of environmental services and crop productivity at different scales [1]. This scenario highlights the imperative need for the development of more sustainable agricultural systems. A business-as-usual attitude towards agricultural production in most world regions will fail to deliver sustainable production intensification to meet future needs [1,2]. Therefore, there is an urgent need for the redesign of agriculture production systems in order to decrease environmental, economic, and social costs associated with current intensive tillage-based production systems that create bare soils and entail high agrochemical applications [3–5].

Conservation agriculture (CA) has been practiced for more than four decades in the pioneer regions in North and South America [1,6]. Based on the positive results obtained, it has been gradually spreading worldwide to address important shortcomings of 'business-as-usual' tillage agriculture in addressing societal needs and environmental challenges. The three interlinked principles that define CA are: (a) continuously minimizing or avoiding mechanical soil disturbance by tillage of all forms, including no inversion of soil layers, and reducing the rate of crop residue break down and avoiding mixing it into the soil, thus preventing short-term peaks of biological activity associated with flushes of carbon (C)

and nitrogen (N) soil inputs and the disruption of soil physical aggregates; (b) maintaining year-round diverse vegetative biomass mulch cover with living and dead plant material over the soil to protect the soil surface and serve as a continuous and diversified source of substrate for a diverse community of soil microorganisms; and (c) enhancing plant species diversification in the cropping system through crop rotations and associations, including cover crops that would utilize any spare time windows between cash crops, including N-fixing legumes which result in a high quality crop biomass input into the soil, enhancing plant-growth promoting bacteria and fungi [1,3,6,7]. Currently, Brazil has about 43 M ha under CA cropland of varying time durations spread across different agro-ecoregions reflecting the continental dimensions of the country (i.e., the fifth largest territorial country in the world in terms of area) and diverse farming traditions [6]. In consequence, there is a complex interaction of weather, soil, and human (CA management experience) conditions creating a mosaic of production management systems. These range from CA systems in the very early years of transition from tillage agriculture to mature CA systems that were transformed by no-till pioneer farmers several decades ago. The consequences for soil health resulting from this range of CA systems of varying duration are not well documented. This is an important knowledge gap that needs be filled in order to avoid drawing the wrong or misleading conclusions about soil quality and CA effectiveness. In this sense, bioindicators, such as soil enzyme activity, are management sensitive, and are strongly associated with the living portion of the soil [8]. Thus, soil enzyme activity can serve as an integrated indicator of various key soil attributes, supporting its assessment as part of a holistic and thorough CA evaluation.

Soil health can be defined as the capacity of a specific soil type to function within natural boundaries in order to sustain plant and animal productivity, maintain or enhance water and air quality, support human and animal health, and incorporate biological diversity [4,9,10]. The concept 'conservation effectiveness' encompasses not only the conservation of soil and water, but also enhancement of the soil biotic component that is the basis of sustainability [1]. In an analogous way, 'crop production effectiveness' encompasses not only the maintenance of topsoil chemical nutrient levels above certain critical levels, but also the provision of a welcoming habitat for diverse soil microbiomes that will stimulate nutrient cycling, soil aggregation, water infiltration and retention, and enhance root uptake of water and plant nutrients.

Soil health requires that the main soil functions, such as productivity capacity, environmental protection, and plant and animal health, are well balanced through wise management decisions [11]. In addition, soil health can be understood as a subcomponent of broader ecosystem health. A healthy ecosystem depends on efficient nutrient cycling, a high photosynthesis rate, continuous energy flow, stability, and resilience to stress [12,13]. In this sense, there is a strong link between ecosystem health, soil health, and plant vigour, in which microbial activity, biodiversity, and community stability play an essential role [13]. Therefore, building soil health through farming practices is a primary pathway for ensuring sustainable agriculture. The microbiome living in the rhizosphere is a hot-spot where the microbiota can act as plant growth promoters and plant growth regulators. Alternatively, the microbiota can act as plant pathogens or plant growth detractors, affecting root growth through negative effects on plant nutrient uptake and water use efficiency, and by exacerbation of biotic and abiotic stress events [14,15]. Building a diverse microbiome in the rhizosphere is necessary to suppress or alleviate pressures from plant pathogens, and to decrease disease incidence and its severity, resulting in more vigorous plants with greater resilience to climate change [13,16,17].

Soil physical attributes, particularly soil texture, structure, compaction, bulk density, aggregation, porosity and water availability, and soil chemical attributes, especially hydrogen potential (pH), soil organic matter (SOM), N, plant root exudates, salinity, aluminium (Al^{3+}), hydrogen, cation exchange capacity (CEC), and cropping system and weather conditions, drive soil microbial activities and their functional diversity [13]. Microbial activity and diversity are sensitive bioindicators of soil management quality [3,15]. Therefore,

assessment of enzyme activity and of the soil microbiome may provide early insights into the quality of soil management in terms of whether it is improving soil health or promoting degradation before matters are too far advanced [13].

Among many soil enzymes that play an important role in soil and plant health, Mendes et al. [8] proposed β-glucosidase and arylsulfatase enzyme activity as key indicators for understanding the function of soil nutrient cycling, assigning similar weights to these enzymes in relation to their importance to this specific function. These two soil enzymes are gradually being included in routine soil analyses in Brazil [8] because they have been found to be efficient bioindicators in the Brazilian Cerrados (Central-West) soils, acting as warning indicators of soil quality changes associated with the production management practices adopted [15,18]. In addition, Mendes et al. have highlighted that these enzymes are strongly associated with crop yields and SOM content [19], fitting well with the need to define critical limits of key bioindicators [18–20]. The β-glucosidase enzyme plays an important role in the soil C cycle and in nutrients cycling, primarily of N [8]. Arylsulfatase plays a role in the soil sulfur (S) cycle, mediating the transformation of organic S to SO_4^{2-}, the form in which it can be taken up by plants. S is an important nutrient in soybean and maize crop nutrition and tropical soils frequently are deficient in its availability to plants [8,21].

The main hypothesis of this study is that crop performance in different yield environments is strongly associated with soil biological activity which can be positively or negatively affected according to a complex interaction involving the soil management system adopted, the soil type, and the weather. A knowledge of this interaction would create opportunities to boost soil life and crop yield.

In this study, we sought to advance knowledge concerning the relationship between varying yield environments (YEs) within production fields and β-glucosidase and arylsulfatase enzyme activity. We also wanted to understand the main soil attributes that affect enzyme activity in the main Brazilian agro-ecoregions. Finally, we wanted to study the presence of plant-growth-promoting organisms and plant pathogenic organisms in the varying YEs. These issues are still only barely covered in the CA literature but knowledge about them would create the opportunity for farmers to apply site-specific soil management to build and sustain soil life and plant health.

The main objective of this study was to assess soil health, through the activity of two enzymes as bioindicators, in long-term CA croplands in the main Brazilian agro-ecoregions. Moreover, in one field for which there was a large temporal data set on crop yield, soil DNA characterization was undertaken to capture microbiome relationship with different crop YEs within field.

2. Materials and Methods

2.1. Agro-Ecoregions, Croplands and Within-Field Yield Environments

This study was carried out in seven grain production fields distributed in four states that have been continuously managed under CA over the past 10 to 20 years. These fields were selected because they offered crop yield records kept by the Aquarius Project team (https://www.facebook.com/projetoaquariusufsm/, accessed on 20 October 2021) which were representative of farming systems currently adopted in their agro-ecoregion. In general, the fields had soybean yields above the national average (3.5 Mg ha^{-1} in 2020/21), with one field (S-1) holding a national record for soybean and maize yields (https://thefurrow.co.uk/brazilian-farmers-aim-for-sustainability/, accessed on 24 October 2021). The selected fields were in the main agro-ecoregions of Brazil which are: South, Central-West ('Cerrado') and Northeast (Figure 1 and Table 1) which have a range of soil textures. The soil type is Hapludox, except in field S-3 which is Paleudalf according to Soil Taxonomy classification. In each field, three within-field YEs were delineated based on crop yield records and satellite images (NDVI) according to the available farm data. The HYE was classified as >110% average crop yield in the field, the MYE as 80–110%, and the LYE as <80%. The criteria to define YEs in the study has been extensively used in precision

agriculture literature [22–25]. In our study, all the previous crop yield data within each field were relativized so that the year and crop grown were taken account of in the study. For example, the maize yield was relativized in relation to the highest maize yield obtained in the field in that specific year taking account of the spatial variability captured through the grain yield sensor fitted in the combine harvester. The same was undertaken for soybean in order to capture the spatial variability in crop performance. Previous studies by the Aquarius Project team have shown that the maize yield map had closer relationships with soil attributes than the soybean yield map, justifying its use in YEs delineation when available. The LYE in each field served as a reference management level that needs to be improved. The HYE in each field and in the whole set of fields served as a reference level of efficient management that needs to be understood in terms of soil attributes that minimize the crop yield limiting factors.

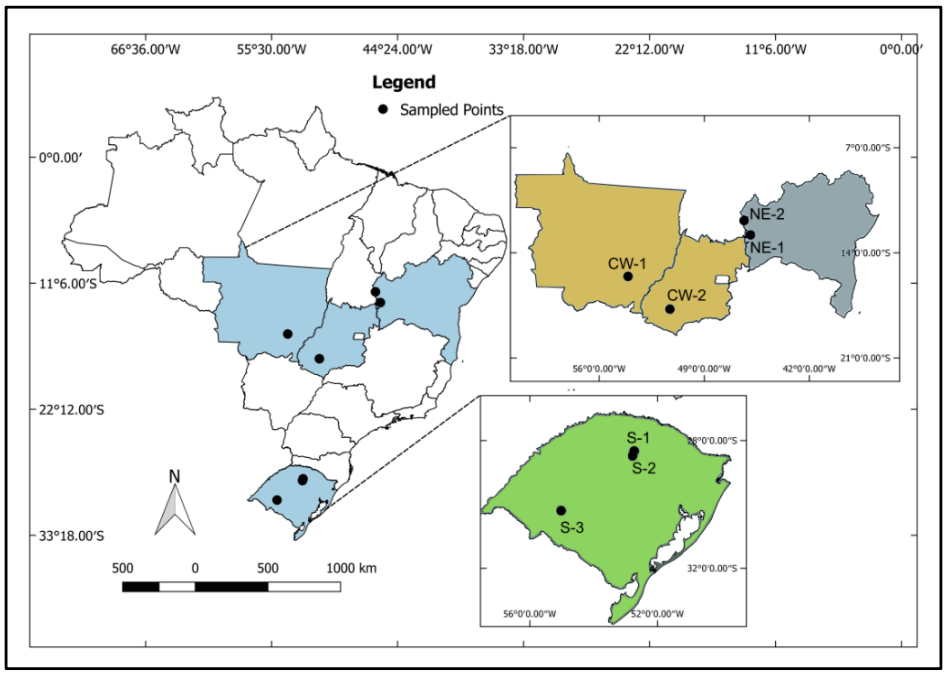


Figure 1. Geographical distribution of the fields sampled in the main agro-ecoregions of Brazil. Agro-ecoregions with different colors: green color = South (fields S-1 = Carazinho, S-2 = Não-Me-Toque and S-3 = Rosário do Sul, in Rio Grande do Sul state); orange color = Central-West (fields CW-1 = Primavera do Leste, in Mato Grosso state and CW-2 = Rio Verde, in Goiás state); gray color = Northeast (fields NE-1 = Luis Eduardo Magalhães and NE-2 = Luis Eduardo Magalhães, in Bahia state).

Table 1. Field locations, areas, average annual temperature (T), annual accumulated precipitation (P), average altitude (E) and soil texture and classification.

Field	Localization	Area	T	P	E	Soil Texture [1]	U.S. Soil Taxonomy [1]
	(City-State)	(ha)	(°C)	(mm y^{-1})	(m)		
S-1	Carazinho-RS	60.1	18.3	1856	565	Clay loam	Typic Hapludox
S-2	Não-Me-Toque-RS	136.0	19.0	1771	500	Clay loam	Typic Hapludox
S-3	Rosário do Sul-RS	25.0	19.5	1493	155	Sandy loam	Paleudalf
CW-1	Primavera do Leste-MT	348.8	24.0	1471	650	Sandy clay loam	Hapludox
CW-2	Rio Verde-GO	509.8	23.1	1294	875	Clay loam	Hapludox
NE-1	Luís Eduardo Magalhães-BA	1376.1	23.6	881	830	Sandy clay loam	Hapludox
NE-2	Luís Eduardo Magalhães-BA	690.9	25.0	1089	880	Sandy clay loam	Hapludox

[1] Soil texture classified according to Soil Survey Staff (2014) [26]; Meteorological data extracted from the database of nearest INMET weather automatic stations, corresponding to the years 2018, 2019, and 2020. RS = Rio Grande do Sul; MT = Mato Grosso; GO = Goiás; BA = Bahia. S = South; CW = Central-West; NE = Northeast.

2.2. Sampling Strategies for Soil Enzyme, Physicochemical Soil Analysis, Soil DNA Characterization and Crop Yield

In each of three YEs, three random samples were collected in the seven fields selected, totaling 63 georeferenced sampling points. The soil was collected at 0–0.10 m depth. Soil samples for enzyme activity analysis were collected 40 days after crop emergence (soybean at flowering stage) using a manual shovel. Seven subsamples were used to obtain one composite soil sample following the scheme: one in the center of crop row and three on each side of the row. After sieving (<2 mm) and removing crop residues, the soil samples were air dried following the methodology proposed by Mendes et al. [8,18]. The β-glucosidase and arylsulfatase enzymes activity laboratory analysis followed the methodology of Tabatabai [27].

The chemical analyses were soil pH in water (1:1), potassium (K^+) and phosphorus (P) extracted with Mehlich-I solution. The K^+ content was determined by flame photometry and the P content by colorimetry, using molybdenum blue [28]. Calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), and Al^{3+} were extracted using 1.0 mol L^{-1} KCl solution [29]. Copper (Cu^{2+}) and zinc (Zn^{2+}) were extracted using HCl 0.1 mol L^{-1} [29]. Ca^{2+}, Mg^{2+}, and Mn^{2+} were determined by atomic absorption spectrophotometry. The Al^{3+} was titrated with NaOH 0.025 mol L^{-1}. S was extracted by Ca phosphate solution and determined in $BaCl_2$ gelatine solution. Boron (B) was extracted by digestion and determined by colorimetry. The CEC at pH 7 was determined by the sum of the exchangeable bases (K^+, Ca^{2+}, and Mg^{2+}) plus potential acidity ($H + Al^{3+}$) according to Tedesco et al. [29]. The soil texture was determined by pipette method according to Teixeira et al. [30].

The SOM content was determined by adapting the Walkley–Black method with oxidation by sulfochromic solution with external heat and Cr^{3+} content by spectrophotometry [29]. The total soil organic C and total N (TOC and TN) concentrations were determined by dynamic flash combustion through elemental analysis (Thermo Fisher Scientific—FlashEA® 1112, Waltham, MA, USA, detection limit = 0.01%). For this, approximately 50 mg of soil, that had been oven-dried (60 °C, 72 h) and finely macerated, was weighed with a scale with 0.001 mg precision and prepared for dry combustion at 975 °C. The TOC and TN stocks were figure out taking account the bulk density of each soil.

The production fields selected for this study had records of soybean and maize yields from previous seasons that allowed the delineation of YEs. During the 2020/21 season the grain yield was determined by manual harvesting of 1 m^2 in three replicates. The weather data were obtained from the nearby weather station of the National Institute of Meteorology (INMET). The normalized differences in vegetation index (NDVI) for soybean were determined keeping the soil enzyme sampling as central points, using the Atfarm® platform (Yara Ltda, Oslo, Norway). The satellite images were selected at soybean flowering stage and classified using an index according to a scale ranging from 0.0 to 1.0.

One of the fields of the Aquarius Project (S-2) that had a large available data set, obtained over a period of 20 years, was selected for soil DNA characterization. The soil texture was clayey, kaolinitic, and classified as a Rhodic Hapludox [26]. This cropland field has been managed under CA management since 2002; more details can be found in Pott et al. [23]. In the growing season of 2019/2020 composite soil samples, following the same methodology previously described, were collected in the HYE, MYE and LYE at 0–0.10 m depth, and sent to Biome Markers® (https://biomemakers.com, accessed on 20 October 2021), in the United States (USA) for molecular analysis of the microbiota. The DNA extraction was performed with the DNeasy 420 PowerLyzer PowerSoil Kit from Qiagen [31]. In order to characterize bacterial and fungal microbial communities associated with the soil samples, the 16S rRNA and ITS marker regions were selected. Libraries were prepared following the two-step PCR Illumina protocol using custom primers amplifying the 16S rRNA V4 region and the ITS1 region as described previously [31]. The DNA sequencing was conducted in an Illumina MiSeq instrument using pair-end sequencing (2 × 300 bp). The platform BeCrop® (West Sacramento, CA, USA) was used in this study, and more details can be found in Imam et al. [31].

In this field the soil penetration resistance in the 0–0.40 m soil depth was determined using a digital penetrometer PLG PenetroLOG 2040 (Falker®, Porto Alegre, Brazil) with 0.01 m depth accuracy. The penetrometer readings were performed at the flowering soybean stage when the soil was close to field capacity with gravimetric soil moisture sampling at 0–0.10, 0.10–0.20, 0.20–0.30, and 0.30–0.40 m [30]. The apparent soil electrical conductivity (EC) was determined close to soil sampling points at 0–0.30 m with VERIS CE® (VERIS Technologies, Salina, KS, USA). The soil macroporosity data from topsoil were obtained from Pott et al. [23].

2.3. Statistical Analysis

The enzyme activity and chemical analysis were submitted to variance analysis ($p < 0.01$ and $p < 0.05$) and Pearson's correlation. In the fields that had soybean grown in the 2020/21 season (54 soil-sampling points), a principal component analysis (PCA) was performed to determine the drivers of soybean yield and enzyme activity and factor analysis was used to better define the original variables that most contributed to the formation of the main components and factors, using the varimax normalized rotation. Additionally, cluster analysis was performed to determine the differences and similarities between agro-ecoregions, fields, and their YEs, using Ward's method for amalgamation (linkage) rule and square Euclidean distances as a measure of similarity. In order to group variables and cases in dendrograms and correlate them with the scatter plot of variables produced in the factorial analysis [32,33], Statistica 12® software was used. The relationships of enzyme activity with soil attributes and biota diversity (number of species identified in the samples) were investigated by linear and quadratic adjustments. The enzyme activity and SOM in each YEs within field was compared by Tukey test ($p < 0.05$). The Tukey test and all relationships used the R Studio® statistical package.

3. Results and Discussion

3.1. Characterization of Soil Attributes, Crop Yield and Enzyme Activity by Yield Environments in Selected Fields

The chemical soil analysis of seven fields selected for this study is presented in Tables 2 and 3. The chemical attributes reflect the farmer's and consultant's management in each agro-ecoregion. Remarkable differences in soil texture ranging from 14% to 39.7% of clay content and 16.7% to 78.1% of sand content were observed (Table 3).

Table 2. Soil contents of phosphorus (P), potassium (K^+), sulphur (S), aluminium (Al^{3+}), calcium (Ca^{2+}) magnesium (Mg^{2+}), cation exchange capacity (CEC), Ca^{2+}/Mg^{2+} ratio, and base saturation (BS) at 0–0.10 m depth in varying yield potential environments (YE) in seven fields managed under conservation agriculture in main Brazilian agro-ecoregions.

Field	YE	P	K^+	S	Al^{3+}	Ca^{2+}	Mg^{2+}	CEC	Ca^{2+}/Mg^{2+}	BS
		(mg dm^{-3})				($cmol_c$ dm^{-3})				(%)
S-1	H	35.5 ± 8.3	308.3 ± 35.8	28.2 ± 15.4	0.0 ± 0.0	6.9 ± 2.0	3.2 ± 0.8	13.5 ± 2.7	2.2 ± 0.1	79.9 ± 3.3
	M	39.1 ± 8.5	327.3 ± 59.0	14.7 ± 2.8	0.0 ± 0.0	5.7 ± 1.2	2.3 ± 0.4	11.2 ± 1.9	2.5 ± 0.4	79.0 ± 0.5
	L	56.8 ± 13.2	205.3 ± 48.6	11.6 ± 0.4	0.0 ± 0.0	4.7 ± 0.9	1.9 ± 0.5	9.2 ± 1.6	2.4 ± 0.1	78.0 ± 1.5
S-2	H	41.3 ± 5.9	385.3 ± 66.4	6.7 ± 1.4	0.0 ± 0.0	5.8 ± 1.3	1.9 ± 0.3	11.5 ± 1.7	3.0 ± 1.0	75.9 ± 5.5
	M	37.4 ± 4.1	293.3 ± 19.9	8.1 ± 0.7	0.0 ± 0.0	5.5 ± 0.5	1.5 ± 0.2	11.3 ± 0.3	3.6 ± 0.3	69.0 ± 5.5
	L	53.2 ± 37.8	283.7 ± 22.0	9.5 ± 0.4	0.0 ± 0.0	5.4 ± 1.2	1.8 ± 0.4	11.4 ± 0.7	3.0 ± 0.3	69.3 ± 5.0
S-3	H	61.3 ± 5.9	236.3 ± 3.8	7.9 ± 3.1	0.0 ± 0.0	9.9 ± 2.5	3.1 ± 0.6	16.2 ± 3.3	3.2 ± 0.0	83.7 ± 2.3
	M	35.8 ± 0.6	125.7 ± 2.3	5.9 ± 0.8	0.0 ± 0.0	8.0 ± 1.3	2.9 ± 0.4	12.7 ± 1.7	2.8 ± 0.1	88.4 ± 1.2
	L	76.7 ± 3.9	246.3 ± 9.1	17.5 ± 4.5	0.0 ± 0.0	3.6 ± 0.2	1.4 ± 0.1	6.8 ± 0.3	2.5 ± 0.0	82.1 ± 0.5
CW-1	H	24.2 ± 7.5	160.3 ± 30.1	18.6 ± 2.9	0.0 ± 0.0	3.3 ± 0.1	1.4 ± 0.1	8.0 ± 0.4	2.3 ± 0.1	65.4 ± 5.2
	M	22.1 ± 7.9	149.0 ± 12.8	16.2 ± 10.4	0.0 ± 0.0	4.0 ± 0.5	1.7 ± 0.3	8.4 ± 0.4	2.3 ± 0.3	72.0 ± 6.8
	L	18.4 ± 9.6	140.0 ± 12.8	13.1 ± 3.4	0.0 ± 0.0	3.9 ± 0.3	1.8 ± 0.3	8.3 ± 0.6	2.2 ± 0.4	73.2 ± 6.6

Table 2. *Cont.*

Field	YE	P	K^+	S	Al^{3+}	Ca^{2+}	Mg^{2+}	CEC	Ca^{2+}/Mg^{2+}	BS
		(mg dm^{-3})				($cmol_c$ dm^{-3})				(%)
CW-2	H	21.1 ± 9.6	135.0 ± 48.6	32.4 ± 16.4	0.0 ± 0.0	5.2 ± 1.7	1.2 ± 0.3	9.7 ± 1.9	4.5 ± 0.4	68.6 ± 8.2
	M	19.3 ± 5.7	142.0 ± 18.4	33.2 ± 16.1	0.0 ± 0.0	5.0 ± 1.2	1.2 ± 0.3	9.9 ± 1.6	4.1 ± 0.1	66.4 ± 6.0
	L	16.2 ± 8.2	178.3 ± 55.5	59.1 ± 18.5	0.0 ± 0.0	5.7 ± 1.1	1.4 ± 0.3	10.0 ± 0.9	4.0 ± 0.5	74.9 ± 5.3
NE-1	H	28.7 ± 6.4	88.3 ± 8.3	8.9 ± 0.7	0.0 ± 0.0	2.7 ± 0.8	1.2 ± 0.4	5.2 ± 1.0	2.3 ± 0.1	78.8 ± 6.7
	M	40.4 ± 15.5	60.3 ± 5.1	8.1 ± 0.6	0.0 ± 0.0	3.0 ± 0.7	1.2 ± 0.2	5.5 ± 0.8	2.4 ± 0.2	79.2 ± 2.4
	L	45.4 ± 10.7	87.3 ± 23.7	7.9 ± 1.6	0.0 ± 0.0	2.7 ± 0.5	1.1 ± 0.3	5.1 ± 0.8	2.5 ± 0.2	77.6 ± 5.1
NE-2	H	48.4 ± 8.5	148.0 ± 34.0	36.6 ± 7.6	0.0 ± 0.0	3.2 ± 0.6	0.9 ± 0.3	5.6 ± 0.9	3.7 ± 0.2	79.2 ± 4.6
	M	62.5 ± 16.7	157.3 ± 38.8	65.6 ± 37.8	0.0 ± 0.0	2.8 ± 0.2	0.6 ± 0.2	4.9 ± 0.4	4.7 ± 0.3	77.9 ± 2.1
	L	54.3 ± 25.0	147.3 ± 54.6	39.6 ± 15.7	0.0 ± 0.0	2.4 ± 0.6	0.5 ± 0.2	4.3 ± 1.0	4.7 ± 0.3	74.0 ± 5.1

S = South (fields S-1 = Carazinho, S-2 = Não-Me-Toque and S-3 = Rosário do Sul, in Rio Grande do Sul State); CW = Central-West (fields CW-1 = Primavera do Leste, in Mato Grosso State and CW-2 = Rio Verde, in Goiás State); NE = Northeast (fields NE-1 = Luis Eduardo Magalhães and NE-2 = Luís Eduardo Magalhães, in Bahia State). H = high yield environment; M = medium yield environment; L = low yield environment. ± = corresponds to the standard deviation.

Table 3. Soil texture, pH in water, potential acidity (H + Al^{3+}), and micronutrients, zinc (Zn^{2+}), cupper (Cu^{2+}), boron (B) e manganese (Mn^{2+}) at 0–0.10 m depth in varying yield potential environments (YE) within fields under conservation agriculture in main Brazilian agro-ecoregions.

Field	YE	pH	H + Al^{3+}	Sand	Silt	Clay	Zn^{2+}	Cu^{2+}	B	Mn^{2+}
			($cmol_c$ dm^{-3})		(%)			(mg dm^{-3})		
S-1	H	6.1 ± 0.1	2.7 ± 0.2	43.2 ± 5.2	18.5 ± 4.1	38.3 ± 1.2	4.3 ± 0.8	3.4 ± 0.5	0.8 ± 0.1	7.0 ± 1.0
	M	6.3 ± 0.3	2.3 ± 0.4	41.7 ± 1.0	23.3 ± 1.2	35.0 ± 2.0	4.5 ± 0.7	3.1 ± 0.2	0.6 ± 0.1	3.3 ± 2.1
	L	6.5 ± 0.1	2.0 ± 0.3	49.7 ± 15.1	22.3 ± 19.0	28.0 ± 5.0	5.5 ± 1.4	2.4 ± 0.1	0.7 ± 0.0	2.3 ± 0.6
S-2	H	6.2 ± 0.2	2.7 ± 0.6	36.7 ± 4.4	25.7 ± 4.7	37.7 ± 0.6	3.7 ± 0.7	2.5 ± 0.4	0.5 ± 0.1	7.0 ± 3.5
	M	6.0 ± 0.2	3.5 ± 0.7	41.5 ± 4.5	19.2 ± 1.8	39.3 ± 3.5	4.1 ± 1.3	3.0 ± 0.9	0.4 ± 0.0	9.7 ± 2.9
	L	5.9 ± 0.3	3.5 ± 0.5	41.6 ± 11.6	18.7 ± 3.7	39.7 ± 10.2	3.3 ± 0.6	2.5 ± 1.0	0.4 ± 0.1	7.3 ± 2.1
S-3	H	6.0 ± 0.0	2.6 ± 0.1	61.9 ± 1.1	18.1 ± 2.8	20.0 ± 1.7	1.4 ± 0.3	0.3 ± 0.0	0.4 ± 0.1	3.7 ± 1.2
	M	6.6 ± 0.1	1.4 ± 0.1	48.4 ± 1.9	33.3 ± 1.1	18.3 ± 1.2	1.1 ± 0.0	0.6 ± 0.0	0.4 ± 0.1	1.7 ± 0.6
	L	6.4 ± 0.0	1.2 ± 0.0	78.1 ± 0.4	7.9 ± 1.4	14.0 ± 1.0	4.2 ± 0.3	0.8 ± 0.1	0.4 ± 0.0	5.3 ± 0.6
CW-1	H	6.2 ± 0.4	2.8 ± 0.6	51.9 ± 2.4	18.7 ± 3.0	29.3 ± 3.1	3.5 ± 0.7	0.8 ± 0.1	0.6 ± 0.2	1.3 ± 0.6
	M	6.5 ± 0.3	2.3 ± 0.6	53.1 ± 3.6	20.9 ± 1.2	26.0 ± 3.0	3.8 ± 0.8	0.9 ± 0.2	0.7 ± 0.2	1.0 ± 0.0
	L	6.6 ± 0.3	2.2 ± 0.7	50.8 ± 1.6	21.2 ± 1.3	28.0 ± 2.6	3.2 ± 0.6	0.8 ± 0.2	0.5 ± 0.1	1.3 ± 0.6
CW-2	H	6.2 ± 0.3	3.0 ± 0.3	27.0 ± 2.4	45.3 ± 6.2	27.7 ± 3.8	7.5 ± 0.9	1.3 ± 0.2	0.7 ± 0.1	1.3 ± 0.6
	M	6.0 ± 0.2	3.3 ± 0.3	23.8 ± 7.2	48.5 ± 10.1	27.7 ± 3.8	10.1 ± 6.6	1.5 ± 0.6	0.6 ± 0.1	2.0 ± 0.0
	L	6.2 ± 0.3	2.5 ± 0.3	16.7 ± 2.6	50.3 ± 3.1	33.0 ± 3.6	2.7 ± 0.8	0.9 ± 0.2	0.7 ± 0.1	1.0 ± 0.0
NE-1	H	7.0 ± 0.5	1.1 ± 0.2	63.9 ± 0.5	10.1 ± 2.1	26.0 ± 1.7	4.0 ± 0.9	1.2 ± 0.7	0.5 ± 0.1	1.0 ± 0.0
	M	6.9 ± 0.1	1.1 ± 0.1	65.9 ± 3.2	9.1 ± 5.1	25.0 ± 2.0	6.2 ± 3.0	1.6 ± 0.6	0.4 ± 0.1	1.0 ± 0.0
	L	7.0 ± 0.2	1.1 ± 0.1	58.3 ± 4.6	12.4 ± 3.6	29.3 ± 4.0	2.7 ± 1.0	0.7 ± 0.3	0.4 ± 0.0	1.0 ± 0.0
NE-2	H	6.5 ± 0.3	1.1 ± 0.3	69.5 ± 3.0	8.5 ± 3.0	22.0 ± 4.4	8.4 ± 0.2	2.3 ± 0.5	0.4 ± 0.0	2.7 ± 2.9
	M	6.7 ± 0.2	1.1 ± 0.2	71.2 ± 4.0	5.4 ± 1.7	23.3 ± 4.0	8.0 ± 0.8	2.1 ± 0.4	0.4 ± 0.1	1.0 ± 0.0
	L	6.6 ± 0.1	1.1 ± 0.0	70.5 ± 0.4	5.8 ± 0.8	23.7 ± 1.2	8.1 ± 3.5	2.2 ± 0.4	0.4 ± 0.1	1.0 ± 0.0

S = South (fields S-1 = Carazinho, S-2 = Não-Me-Toque and S-3 = Rosário do Sul, in Rio Grande do Sul State); CW = Central West (fields CW-1 = Primavera do Leste, in Mato Grosso State and CW-2 = Rio Verde, in Goiás State); NE = Northeast (fields NE-1 = Luis Eduardo Magalhães and NE-2 = Luís Eduardo Magalhães, in Bahia State). H = high yield environment; M = medium yield environment; L = low yield environment. ± = corresponds to the standard deviation.

The soybean and corn yield (NE-2), NDVI, β-glucosidase and arylsulfatase soil enzymes activity, TN, TOC and SOM are shown in Table 4. The SOM had a large range of 1.4% to 4.2%, and so as did TOC and TN. These results were associated with cropping systems, soil texture, and climate of different agro-ecoregions. The β-glucosidase ranged from 90.8 to 256.0 mg p-nitrophenol kg^{-1} soil h^{-1} and arylsulfatase from 32.3 to 287.3 mg p-nitrophenol kg^{-1} soil h^{-1}. This broad range provided us with the opportunity to explore the relationships of soil attributes, crop yield, and enzyme activity (Table 4).

Table 4. Soybean and corn* grain yield near soil and enzyme sampling, normalized difference vegetation index (NDVI), β-glucosidase e arylsulfatase soil enzyme activity, soil organic matter (SOM), total soil organic carbon (TOC), total nitrogen (TN) and carbon/nitrogen ratio (C/N), and at 0–0.10 m depth in varying yield potential environments (YE) in same field managed under conservation agriculture in main Brazilian agro-ecoregions.

Field	YE	Crop Yield	NDVI	β-Glucosidase	Arylsulfatase	SOM [1]	TOC [2]	TN [2]	C/N
		(kg ha^{-1})		(mg p-Nitrophenol kg^{-1} soil h^{-1})		(%)	(Mg ha^{-1})		
S-1	H	5532 ± 1210	0.90 ± 0.02	209.8 ± 25.5	287.3 ± 8.1	3.2 ± 0.12	27.6 ± 1.97	2.36 ± 0.15	11.7 ± 0.23
	M	4514 ± 287	0.73 ± 0.05	180.0 ± 23.7	259.5 ± 2.4	2.8 ± 0.38	23.8 ± 1.83	2.06 ± 0.17	11.6 ± 017
	L	4582 ± 288	0.43 ± 0.10	205.2 ± 7.8	235.9 ± 2.7	2.7 ± 0.06	19.8 ± 1.15	1.71 ± 0.10	11.5 ± 0.26
S-2	H	6120 ± 106	0.88 ± 0.03	210.5 ± 18.3	253.5 ± 17.1	3.3 ± 0.00	26.5 ± 1.60	2.25 ± 0.16	11.8 ± 0.15
	M	5686 ± 733	0.74 ± 0.04	198.3 ± 27.3	240.4 ± 47.8	3.2 ± 0.32	26.7 ± 1.67	2.27 ± 0.29	11.8 ± 0.73
	L	5144 ± 268	0.31 ± 0.06	215.4 ± 10.8	249.8 ± 53.5	3.1 ± 0.25	25.4 ± 1.65	2.08 ± 0.18	12.2 ± 0.44
S-3	H	4530 ± 570	0.77 ± 0.08	213.0 ± 12.0	255.7 ± 54.8	2.7 ± 0.00	23.5 ± 0.42	2.14 ± 0.08	11.0 ± 0.37
	M	3620 ± 151	0.55 ± 0.05	183.0 ± 4.3	222.5 ± 19.8	2.3 ± 0.06	20.8 ± 0.98	1.73 ± 0.04	12.0 ± 0.27
	L	3600 ± 60	0.38 ± 0.02	119.4 ± 5.9	78.6 ± 8.6	1.7 ± 0.00	15.1 ± 1.88	1.52 ± 0.18	9.9 ± 0.13
CW-1	H	3629 ± 158	0.69 ± 0.12	162.1 ± 16.6	91.8 ± 6.2	3.3 ± 0.06	27.7 ± 1.65	1.90 ± 0.15	14.6 ± 0.28
	M	3627 ± 289	0.59 ± 0.12	136.7 ± 27.5	70.6 ± 6.7	3.1 ± 0.26	24.6 ± 1.96	1.75 ± 0.15	14.1 ± 0.11
	L	3932 ± 159	0.17 ± 0.08	130.0 ± 20.3	68.1 ± 7.8	3.1 ± 0.44	27.2 ± 1.37	1.87 ± 0.13	14.6 ± 0.40
CW-2	H	4711 ± 197	0.79 ± 0.13	207.4 ± 6.2	160.2 ± 12.2	3.9 ± 0.40	36.3 ± 5.13	2.66 ± 0.48	13.7 ± 0.63
	M	4496 ± 360	0.55 ± 0.19	233.0 ± 29.5	199.3 ± 47.6	3.8 ± 0.12	35.3 ± 4.82	2.63 ± 0.39	13.4 ± 0.20
	L	4032 ± 393	0.28 ± 0.08	256.0 ± 12.9	232.7 ± 10.6	4.2 ± 0.47	36.9 ± 4.05	2.74 ± 0.37	13.5 ± 0.33
NE-1	H	5443 ± 193	0.87 ± 0.15	157.1 ± 1.2	49.4 ± 13.1	1.3 ± 0.10	12.1 ± 0.95	1.01 ± 0.14	12.1 ± 0.80
	M	4956 ± 283	0.80 ± 0.18	140.4 ± 36.6	48.3 ± 12.9	1.7 ± 0.21	13.6 ± 2.20	1.10 ± 0.30	12.6 ± 1.56
	L	5042 ± 416	0.41 ± 0.04	112.8 ± 13.5	32.6 ± 6.8	1.5 ± 0.23	13.1 ± 3.83	0.85 ± 0.22	15.3 ± 0.73
NE-2 *	H	11,333 ± 885	0.80 ± 0.03	133.3 ± 24.4	28.2 ± 3.3	1.5 ± 0.15	11.7 ± 2.45	0.89 ± 0.20	13.3 ± 0.79
	M	12,442 ± 282	0.74 ± 0.03	121.8 ± 18.5	38.2 ± 12.3	1.4 ± 0.26	11.0 ± 3.27	0.80 ± 0.22	13.6 ± 0.50
	L	12,827 ± 558	0.53 ± 0.06	90.8 ± 11.4	32.3 ± 4.9	1.4 ± 0.29	12.9 ± 2.50	0.94 ± 0.23	13.9 ± 0.78

* Field cultivated with maize. S = South (fields S-1= Carazinho, S-2 = Não-Me-Toque and S-3 = Rosário do Sul, in Rio Grande do Sul State); CW = Central-West (fields CW-1 = Primavera do Leste, in Mato Grosso State and CW-2 = Rio Verde, in Goiás State); NE = Northeast (fields NE-1 = Luis Eduardo Magalhães and NE-2 = Luís Eduardo Magalhães, in Bahia State). H = high potential yield environment; M = medium potential yield environment; L = low potential yield environment. [1] SOM determined by wet oxidation through the Walkley–Black adapted method [29]; [2] TOC and TN determined by dry combustion method. ± = corresponds to the standard deviation.

3.2. Soil Attributes by Fields in Agro-Ecoregion and Relationship with Soil Enzyme Activity

According to agro-ecoregion, the soil attributes showed differences that were related to soil type and soil fertility management (Table 2). Soil texture affected soil enzyme activity in the South and Central-West agro-ecoregions but not in the Northeast (Table 5). In general, in the South and Central-West agro-ecoregions the increase of sand content was associated, as expected, with a decrease in enzyme activity. On the other hand, in the Northeast, where the soils had high sandy content and there was a narrow range in soil texture (Table 3), it was not observed. Soil texture influenced structure, CEC, SOM content, soil temperature, and water holding capacity that affect the biological activity in soil. Typically, clay soils are expected to have higher microbial biomass and enzyme activity than sandy soils under similar weather and management conditions.

Ji et al. [34] reported that the actinomyces and fungi population in clay soil was 151% and 43% higher than in loam soil. The authors linked this result to fine clay particles that hold higher water content and SOM than sand and silt particles. Elliot et al. and Alvarez et al. [35,36] have highlighted the protective effect of clay on the microbiome. In our study the clay content had a relationship with β-glucosidase in the South fields, and with arylsulfatase in the South and Central-West fields (Table 5).

Table 5. Pearson's correlation of β-glucosidase and arylsulfatase with soil organic matter (SOM), total nitrogen (TN), soil texture, cation exchange capacity (CEC), and calcium content (Ca^{2+}) in fields in ecoregions.

	Fields in Agro-Ecoregion	SOM [1]	TN [2]	Sand	Silt	Clay	CEC	Ca^{2+}
		(%)	(Mg ha^{-1})		(%)		($cmol_c$ dm^{-3})	
β-glucosidase (mg p-nitrophenol kg^{-1} soil h^{-1})	South	0.78 **	0.72 **	−0.61 **	0.39 *	0.48 *	0.49 *	0.35 ns
	Central-West	0.83 **	0.80 **	−0.91 **	0.85 **	0.43 ns	0.58 *	0.56 *
	Northeast	0.67 **	0.36 ns	−0.07 ns	0.24 ns	−0.13 ns	0.31 ns	0.20 ns
	Average	0.77 **	0.81 **	−0.76 **	0.70 **	0.41 **	0.67 **	0.59 **
Arylsulfatase (mg p-nitrophenol kg^{-1} soil h^{-1})	South	0.79 **	0.70 **	−0.72 **	0.35 ns	0.67 **	0.55 **	0.38 ns
	Central-West	0.80 **	0.74 **	−0.89 **	0.82 **	0.47 *	0.51 *	0.53 *
	Northeast	−0.13 ns	0.19 ns	−0.08 ns	−0.06 ns	0.18 ns	−0.14 ns	−0.18 ns
	Average	0.65 **	0.73 **	−0.64 **	0.49 **	0.53 **	0.82 **	0.72 **
SOM	South	-	0.89 **	−0.78 **	0.24 ns	0.84 **	0.37 ns	0.13 ns
	Central-West	-	0.92 **	−0.83 **	0.81 **	0.26 ns	0.72 **	0.75 **
	Northeast	-	0.61 **	−0.29 ns	0.30 ns	0.13 ns	0.54 *	0.38 ns
	Average	-	0.95 **	−0.86 **	0.78 **	0.49 **	0.61 **	0.46 **

[1] SOM determined by wet oxidation through the Walkley–Black adapted method [29]; [2] TN determined by automated dry combustion method. Significance codes: ** $p < 0.01$; * $p < 0.05$; ns = not significant; $n = 63$.

The CEC had a positive effect on enzyme activity in the South and Central-West fields. In tropical soils, the CEC is dependent on clay mineralogy and is mainly of SOM content. Soares et al. and Bayer et al. [37,38] reported that Oxisols, which are highly weathered, had around 80% of their CEC associated with SOM content. The interaction between SOM and clay minerals (i.e., organomineral complexes) increases soil aggregation and physically protects SOM from microbial degradation. Ferreira et al. and Xu et al. [39,40] reported that CEC and base saturation (BS) were drivers of SOM content in tropical CA soils. These results indicate that nutrient management plays an important role in SOM restoration in dystrophic tropical Oxisols.

In our study, the Ca^{2+} content had a positive relationship with enzyme activity in the Central-West fields (Table 5). In addition, averaged across all fields, Ca^{2+} had relationships with β-glucosidase and arylsulfatase enzyme activity of 0.59 and 0.72 ($p < 0.01$), respectively (Table 4). In the South and Central-West fields, there was no significant relationship with Ca^{2+} content. This result could be explained as follows: In the Central-West fields the Ca^{2+} content was in the range 3.3 to 5.7 $cmol_c$ dm^{-3}. In this range crop yield has a high probability of increasing with further Ca^{2+} input. In the South fields most of the Ca^{2+} content values were already higher than 5.5 $cmol_c$ dm^{-3}, so that the probability for crop yield to increase with further input of Ca^{2+} is low. On the other hand, in the Northeast region fields most of the Ca^{2+} content values were low, in a narrow range from 2.4 to 3.2 $cmol_c$ dm^{-3}, that could explain the lack of a clear relationship (Table 2). Previously, Pires et al. [7] reported that Ca^{2+} was a driver of β-glucosidase in a South CA long-term experiment. Ca^{2+} serves as a constituent of plant cell walls and membranes and can act as a physical barrier against pathogens [41]. It is assumed that a healthy plant provides higher amounts of exudates to feed the soil biota. In addition, Ca^{2+} increases root growth, mainly of the finer roots that are very active in providing exudates to the microbial rhizosphere community. Finally, Ca^{2+} is important for soil aggregation and SOM stabilization under CA [39] and the increase of Ca^{2+} content in subsoil alleviates the Al^{3+} toxicity [42] boosting plant root growth and, in consequence, microbial activity.

The SOM had a stronger relationship with enzyme activity in the South and Central-West fields with r values of 0.78 to 0.83 ($p < 0.01$) (Table 5). In the Northeast fields the SOM had a relationship with β-glucosidase but not with arylsulfatase. Moreover, in this agro-ecoregion the only soil attribute that had a relationship with β-glucosidase activity was SOM. The relationship of β-glucosidase and arylsulfatase enzyme activity with TOC was previously reported by Mankolo et al. [43] in Alabama (USA) cotton fields, with r values

of 0.58 and 0.66, respectively, and with TN, with *r* values of 0.39 and 0.48, respectively. These authors highlight that the enzymes β-glucosidase and arylsulfatase were efficient bioindicators in detecting changes in soil tillage systems (CA compared to conventional tillage). The sandy soils from Alabama in that study had a soil texture similar that of the soils in the Central-West fields in our study (Table 5).

In our study, TN had relationships of 0.94, 0.81, and 0.73 ($p < 0.01$) with SOM, β-glucosidase, and arylsulfatase enzymes, respectively (Table 5). These relationship r values are higher than those reported by Mankolo et al. [43]. In our study, the lower relationship between TN and SOM with enzyme activity occurred in the Northeast fields, where only the β-glucosidase had a significant relationship with SOM. This result may be associated with the lower lability of SOM in this agro-ecoregion fields that had higher C/N ratio (12.0 to 15.4) than other agro-ecoregions fields, such as the South that had lower C/N (9.9 to 12.2) (Table 4).

In the PCA analysis, the two main components explained 92.2% of the data variance of enzyme activity, physics, and chemical soil attributes, elevation, precipitation, temperature, NDVI, and crop yield (Figure 2a). Attributes that related positively to enzyme activity were SOM, TOC, TN, CEC, Ca^{2+} content, and Ca^{2+}/Mg^{2+} ratio and $H + Al^{3+}$. On the other hand, in the opposite quadrant (QIV), sand content, soil pH in water, pH SMP index, temperature, and Mg^{2+} saturation were negatively related to enzyme activity (Figure 2a,b). Regarding soybean yield, the main drive factors were NDVI, yield obtained in previous seasons, clay content, and Cu^{2+} and Mn^{2+} micronutrients content. In general, it was possible to note that a greater number of attributes were associated with enzyme activity (QII Figure 2a). This result confirms that enzyme activity is a sensitive environmental bioindicator.

The annual average air temperature was positioned in the opposite quadrant (QIV Figure 2a) from enzyme activity (QII Figure 2a). The high temperature typical of the tropical environment enhanced SOM biological oxidation rate and reduced its stock, mainly the labile fraction, that is the main substrate for soil biota. On the other hand, the annual average precipitation was positioned in the same quadrant of enzyme activity (QII Figure 2a). The high precipitation could be associated with a high photosynthesis rate and high crop residue input resulting in higher SOM content and enzyme activity. In addition, microbiota is positively related to increase in soil moisture until it reaches a maximum limit [39].

The dispersion case is shown in Figure 2b where the Northeast agro-ecoregion field was positioned in quadrant QIV, being in a distinct position relative to other fields. The Northeast fields have some specific characteristics that may explain this fact, such as higher air temperature, lower annual precipitation (Table 1), higher sand content, lower SOM content, higher soil pH in water and pH SMP index, and a narrower Ca^{2+}/Mg^{2+} ratio (Table 2) in relation to the other fields. In general, the South and CW-1 fields were positioned in the same quadrants (QI and QII). These fields have distinct characteristics of climate and soil according to the agro-ecoregions they are located in, but they have in common the fact that the high quality CA systems have gradually improved soil quality based on the increase in crop residue input by use of cover crops (i.e., consortium of cover crops in South agro-ecoregion field) or tropical pasture with deep root systems (e.g., *Brachiaria* in single use or in combination with maize in CW-2 agro-ecoregion field). In addition, there was an improvement in subsoil chemical quality by Ca^{2+} increase and Al^{3+} decrease and site-specific soil fertility management through precision agriculture, resulting in these fields being grouped in the same quadrants in the PCA analysis.

The CW-1 field was positioned in a quadrant different from most of the other fields (QIII Figure 2). The CW-1 field had higher sand content (Table 2), lower SOM content (Table 3), and lower elevation (Table 1) in relation to the CW-2 field, thus helping to explain that although both sites were in the Central-West agro-ecoregion, they were positioned in different quadrants. The S-3 agro-ecoregion (Rosário do Sul county) had sandy textured soil, lower altitude, lower precipitation, and higher summer temperature (Table 1) that resulted in lower SOM content (Table 4) in relation to the S-1 and S-2 fields (Não-Me-Toque and Carazinho counties, respectively). The LYE of the S-3 field was positioned in the same

quadrant as the CW-1 field (QIII). In general, most of the S-1 and S-2 fields with MYE and HYE were positioned in quadrant QI that was associated with high crop yield in the PCA (QI Figure 2a). Most of the S-1, S-2 and CW-2 fields with MYE and LYE were positioned in quadrant QIII that was associated with enzyme activity in the PCA analysis (QII).

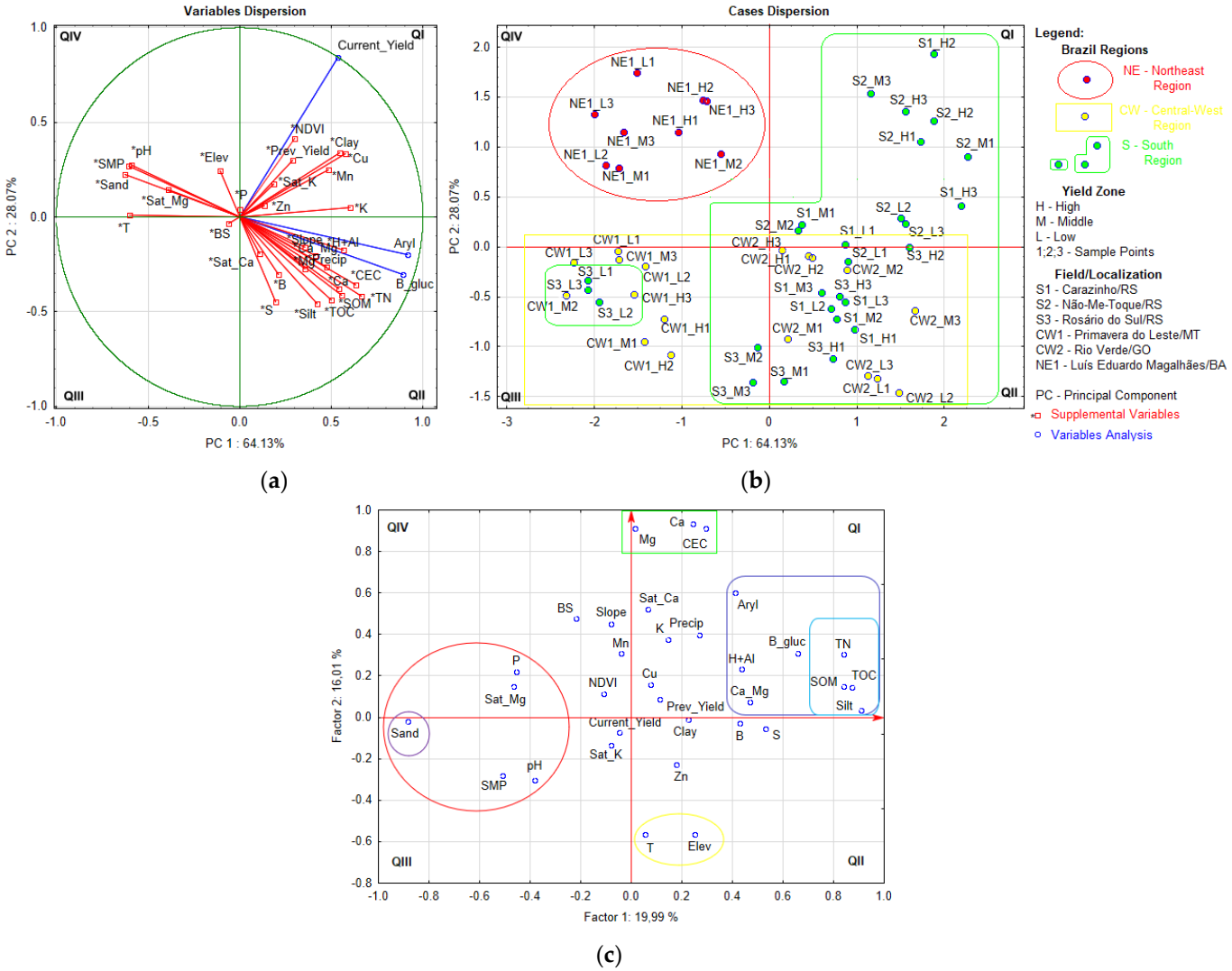


Figure 2. (**a**) Projection of the dispersion of variables (**b**) and of the cases by principal components analysis (PCA), among soil attributes, elevation, climate and crop yield in conservation agriculture fields of main Brazilian agro-ecoregions. β-glucosidase (B_gluc) and arylsulfatase (Aryl) activity and soybean crop grain yield (Current_Yield) of the 2020/21 season were taken as the main variables in the analysis. The supplementary variables (*) were: previous years relative yield (Prev_yield); sand; silt; clay; total nitrogen (TN); total organic carbon (TOC); potential of hydrogen (pH); SMP index (SMP); phosphorus (P); potassium (K); soil organic matter (SOM); Ca^{2+}; Mg^{2+}; cation exchange capacity (CEC); potential acidity (H+Al); bases saturation (BS); sulphur (S); Zn^{2+}; Cu^{2+}; boron (B); Mn^{2+}; calcium/magnesium relation (Ca_Mg); calcium saturation (Sat_Ca); magnesium saturation (Sat_Mg); potassium (Sat_K); average annual air temperature (T); annual precipitation (Precip); slope; elevation (Elev); normalized difference vegetation index (NDVI); (**c**) dispersion of variables, according to their contribution to the formation of Factors 1 and 2; n = 54.

Interestingly, even the fields with very distinct soil attributes, such as soil texture and climate such as subtropical (S-3) and tropical (CW-1), were grouped together with regards to crop performance and enzyme activity (S and CW-1 fields in quadrant QII and S-3 LYE and CW-2). This suggests that soil management had a strong effect on crop performance and enzyme activity, regardless the climate and soil texture. This result suggests that with appropriate regional management practices under CA, it is possible to reach high crop

performance and soil health, assessed by enzyme activity in a broad range of soil types and climates. The case of the S-3 agro-ecoregion is particularly interesting in LYE which was closer to the CW-1 field (QIII), in MYE which was closer to the CW-2 field (low portion of the QII), and finally, in HYE which was closer to the S-1 and S-2 fields in MYE or LYE and with the CW-2 field in HYE (high position of quadrant QII). In decreasing order of crop performance and enzyme activity, we observed: S-1 > S-2 > S-3 > CW-2 > CW-1 > NE-1. It is important to highlight that S1 is the field that had Du Pont national prizes for high soybean yield (5668 kg ha^{-1} in the 2015 season) (https://revistacultivar.com.br/noticias/dupont-do-brasil-reconhece-desempenho-de-sojicultores-da-regiao-sul, accessed on 20 october 2021) and in 2016/17 season the maize yield in this field reached 14,700 kg ha^{-1}. These cases demonstrate that CA management was a tool that facilitated higher crop yields with improved soil life based on enzyme activity and restoration of SOM content (Table 4).

The soil attributes and weather attributes in the PCA analysis are shown in Figure 2c. In the first quadrant (QI), the arylsulfatase and β-glucosidase were grouped, as expected, with SOM, TOC, TN and silt content (Table 3) and Ca^{2+}/Mg^{2+} ratio (Table 2). In addition, the CEC, Ca^{2+} and Mg^{2+} content and Ca^{2+} saturation, annual precipitation, K^{+} and Cu^{2+} content, and previous crop yields were positioned in Q1, i.e., the same quadrant of high crop performance (Figure 2a). In an opposite position to these attributes were grouped sand content, Mg^{2+} saturation, soil pH in water and pH SMP (QIII and QIV, Figure 2c). It should be highlighted that in the NE-1 agro-ecoregion the soil pH in water was higher than in other agro-ecoregion fields (Table 3) justifying in part the presence of this attribute. While the Mg^{2+} saturation was associated with imbalances in the Ca^{2+}/Mg^{2+} ratio. On the other hand, the P content that had values within the range 18.4–76.7 mg dm^{-3} show that some fields had very high P content concentrate in a specific soil layer (0-0.10 m), where a content near to 15 mg dm^{-3} could be the crop critical level (Table 2).

The temperature and elevation (QII, Figure 2c) were also grouped in a position far from the promoters of crop performance confirming that the high temperature in the tropical environment was an important plant abiotic stress factor.

Figure 3 shows the relationship of key soil attributes with β-glucosidase and arylsulfatase. This figure shows that SOM content in 0–0.10 m depth had a positive linear relationship with β-glucosidase which explained around 60% of the variability of this enzyme activity. The maximum enzyme activity was reached with the highest SOM content (near to 5%). The arylsulfatase had a quadratic relationship with SOM, with maximum activity reaching close to 3.55%. Xu et al. [40] reported that SOM and TN had a positive relationship of 0.83 with enzyme activity. The authors explained that microorganisms need nutrients and energy from labile fractions of SOM. In addition, SOM retains soil moisture, and enhances CEC and soil aggregation that boosts microbial activity. In our study TN had a positive linear relationship with β-glucosidase suggesting that legume cover crops could be an important strategy to restore TN stocks and enhance soil biological activity [38].

A recent exploratory study of soil analyses from South Brazil laboratories (n = 35,362) reported that 55% of the soil samples had SOM < 2.5% [44]. In our study, with a more limited database, we found around 40% of the data points with low SOM (<2.5%) that were associated with low enzyme activity (Figure 3). These data suggest an urgent need to revise the cropping system adopted by enhancing rotations and the use of legume cover crops in association with no tillage in order to build up SOM [45].

SOM restoration and enzyme activity are strongly linked to CA principles. Pires et al. [7] reported in a long-term tillage systems experiment (32 years) that crop rotation and cover crops under CA increased SOM in the topsoil compared with intensively tilled soils. Moreover, the crop diversification increased soil microbial diversity, resulting in aggregate stability and SOM protection. In their study, the β-glucosidase activity was 69% higher in CA than in tillage-based systems. Moreover, β-glucosidase was increased by 23% under CA with crop rotation compared to no-till monocropping systems. Avoidance of mechanical soil disturbance stimulates fungi growth and its hyphal networks, which allows fungi to establish bridges at the mulch–soil interface, increasing SOM stabilization. In addition,

the effect of maintaining the mulch cover year-round in the range of 3–5 Mg ha^{-1} on the soil surface is to reduce soil temperature and increase soil moisture that enhances beneficial fungi activity and balances the fungi/bacteria ratio (F/B) [46]. In general, the fungi community is more sensitive to soil disturbance and the quantity and quality of root exudates and crop residues input [47]. Therefore, this community can be boosted by polyculture of cover crops including legumes and crop rotation [48,49].

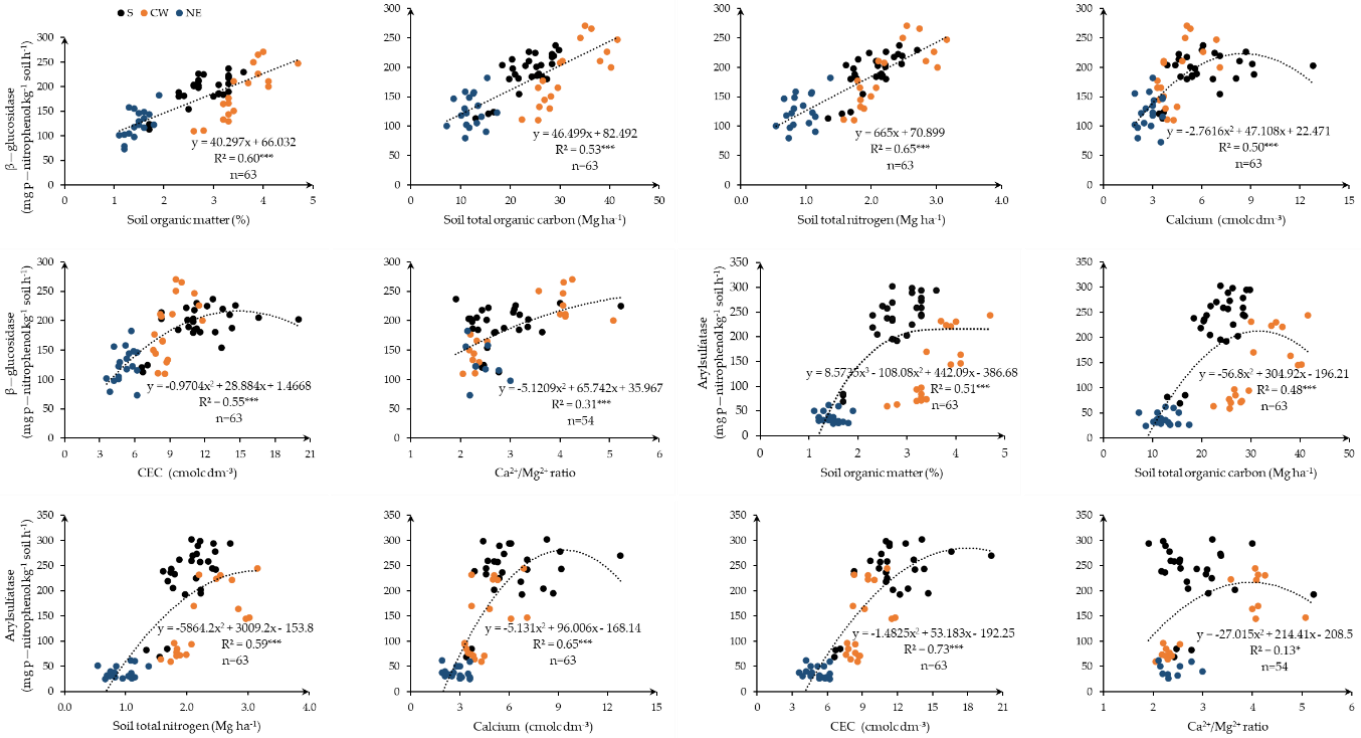


Figure 3. Relationships between the activity of β-glucosidase and arylsulfatase enzymes with soil organic matter (SOM) determined by wet oxidation, total organic carbon (TOC), and nitrogen (TN) determined by dry combustion, calcium content (Ca^{2+}), cation exchange capacity (CEC) and Ca^{2+}/Mg^{2+} ratio in seven Brazilian fields from main agro-ecoregions. Significance codes: *** $p < 0.001$; * $p < 0.05$.

The Ca^{2+} content had a quadratic relationship with β-glucosidase reaching a plateau near 8 $cmol_c$ dm^{-3} that is double the Ca^{2+} critical level suggested for most of crops (4 $cmol_c$ dm^{-3}). The CEC had a quadratic relationship with β-glucosidase reaching the plateau near 14 $cmol_c$ dm^{-3}. The Ca^{2+}/Mg^{2+} ratio also had a quadratic relationship with β-glucosidase suggesting that a ratio of 3–5 could boost this enzyme activity. The fields with Ca^{2+}/Mg^{2+} below 3 and Mg^{2+} saturation higher than 20% were associated with long-term dolomitic lime input [42] and monocropping soybean that had higher Ca^{2+} grain exportation than Mg^{2+} [50].

Dalla Nora and Amado [51] reported that Ca^{2+} and Mg^{2+} contents are drivers of root growth in acid tropical soils allowing roots access to available water in the subsoil. The positive effect of Ca^{2+} in root cell division results in higher root growth which is important for SOM content restoration and improvements in soil aggregation in addition to increasing root exudates that stimulate soil biota and enzyme activity [39].

The arylsulfatase had a quadratic relationship with SOM, TOC and TN, having lower sensitivity to these attributes than β-glucosidase that had a linear relationship with these attributes. The arylsulfatase had a quadratic relationship with Ca^{2+} content but with higher sensitivity to low Ca^{2+} content than β-glucosidase (Figure 3). A similar trend was noted with CEC where arylsulfatase was more sensitive than β-glucosidase to low CEC values. The Ca^{2+}/Mg^{2+} ratio was more critical to β-glucosidase than to arylsulfatase, although both had quadratic relationships.

The P content was grouped with other attributes that were contrary to crop yield and enzyme activity (Figure 2c). This behavior was not expected in weathered tropical soils with high P-fixation capacity. However, long-term high rates of P fertilization input with high rates at the same depth could result in strong vertical nutrient stratification due to low P soil mobility [52,53] with a negative impact on soybean root deepening and grain yield under water stress [54]. This is because there is a strong stimulus to shallow root growth in high P concentration zones in detriment to deepening of the root system through soil profile (Table 2). Previous studies aimed at evaluating the relationship of soil P content and soil biota have revealed that high P content was associated with reduction in biota diversity, mainly mycorrhizal fungi [55–57]. In addition, excessive P fertilization can aggravate Zn^{2+} and Cu^{2+} deficiency, as noted in this study in Figure 4 and in the PCA analysis (Figure 2c).

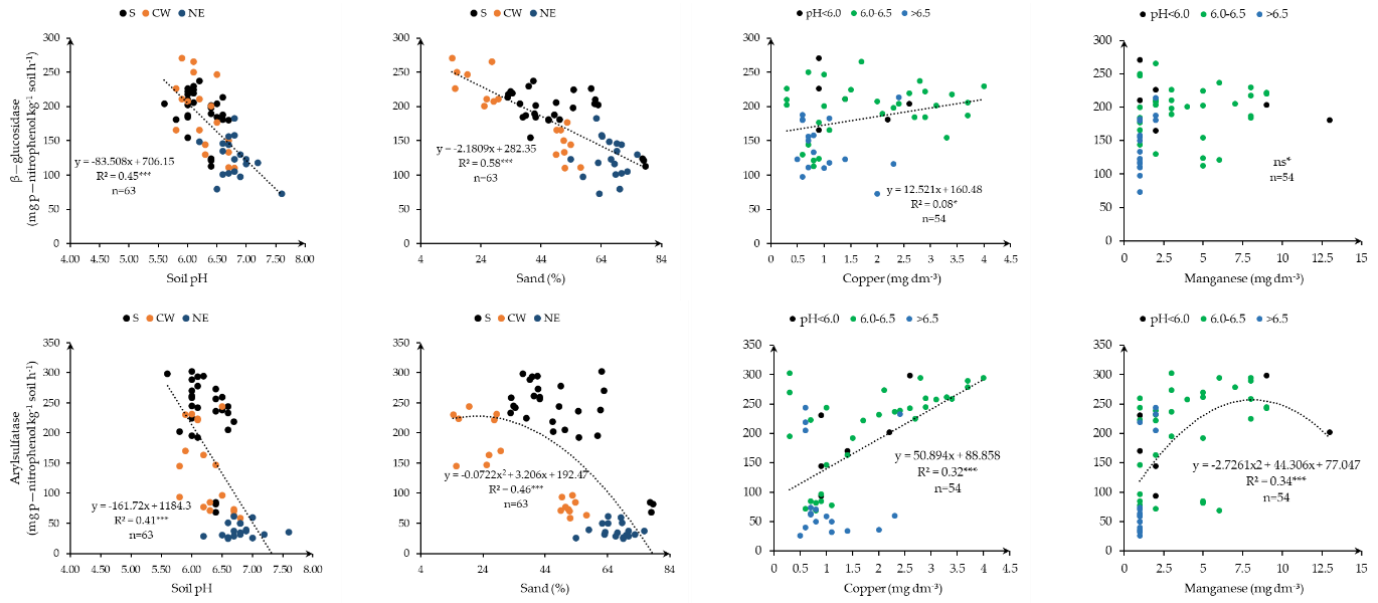


Figure 4. Relationships among the activity of β-glucosidase and arylsulfatase enzymes with soil pH, sand content, copper (Cu^{2+}) and manganese (Mn^{2+}) in seven Brazilian fields from main agro-ecoregions. Significance codes: *** $p < 0.001$; * $p < 0.05$; ns* = not significant.

Figure 4 shows the relationship of soil pH water and sand texture with enzyme activity, where a negative linear relationship was found. The negative relationship of soil pH in water with enzyme activity in acid tropical and subtropical soils was not expected, but was associated with values of soil pH in water reported in our study (Table 2). The range of soil pH in water was 5.9 (±0.3) to 7.0 (±0.5) with a high number of sampling points with soil pH in water exceeding 6.0 that could explain the negative relationship found. In Table 2 only 9.5% of the sampling points were less than 6.0 soil pH in water where a positive relationship between soil pH in water and enzyme activity was expected. On the other hand, 46.0% had higher values than 6.5 soil pH in water where the probability of lime crop yield response was very low.

Although, both enzymes investigated had a negative linear relationship with high soil pH in water, the linear coefficient for arylsulfatase was almost double that of β-glucosidase, suggesting that the former was more sensitive to change in soil pH in water. However, values of soil pH in water higher than 6.5 in acid tropical soils were harmful to enzyme activity. In natural conditions, these soils generally have a soil pH in water below 5.0 [58]. Although the microbiome has mechanisms to adjust to abrupt environmental changes from an acid soil pH in water to much higher levels than in the natural condition, this can cause breakages in the helical DNA structure and increase in lipid hydrolysis in microorganisms [59–61].

Stark et al. [62], in tundra ecosystems, reported that an increase in soil pH in water from 5.6 to 6.6 resulted in higher plant nutrient availability which in turn enhanced enzyme activity, mainly β-glucosidase. However, when the soil pH in water was increased above 6.6, there was a decrease in enzyme activity as reported in our study. However, there is some uncertainty about whether this was a direct effect on microbiota or on the available organic substrate. In our study the optimum range of pH for enzyme activity was in the range of 5.5 to 6.5.

The Northeast field had the highest soil pH in water values (Figure 2). The soil of this agro-ecoregion is sandy with low SOM content that results in a low buffer capacity. Many farmers from that agro-ecoregion come from the South agro-ecoregion and they are used to applying higher lime rates adjusted to clay soils and high SOM content and, in consequence, to higher buffer capacity [63]. The input of these rates in the Northeast fields resulted in a sharp increase in soil pH in water that was associated with low metallic micronutrients availability, such as Cu^{2+} and Mn^{2+} [64] (Figure 4). The sand content had a linear and quadratic relationship with β-glucosidase and arylsulfatase, respectively. As the sand texture increased, the SOM and TN decreased (Table 5) with a negative impact on the soil biota. In our study a positive relationship of Cu^{2+} with both enzyme activity and of Mn^{2+} to arylsulfatase activity was found. The metal organic complex of Cu^{2+} and Mn^{2+} affects the plant nutrient uptake [65] and the enzyme activity (Figure 4). This agrees with the PCA analysis that showed that these metallic micronutrients were associated with crop yield in previous seasons and NDVI in the current season (Figure 2). Considering that the low metallic micronutrients availability was associated with soil pH in water higher than 6.5 highlights that there is a need in some tropical soils with low buffer capacity to accurately formulate the lime rate prescription and use fertilizers with these nutrients to compensate for their removal in grain exportation (Figure 4).

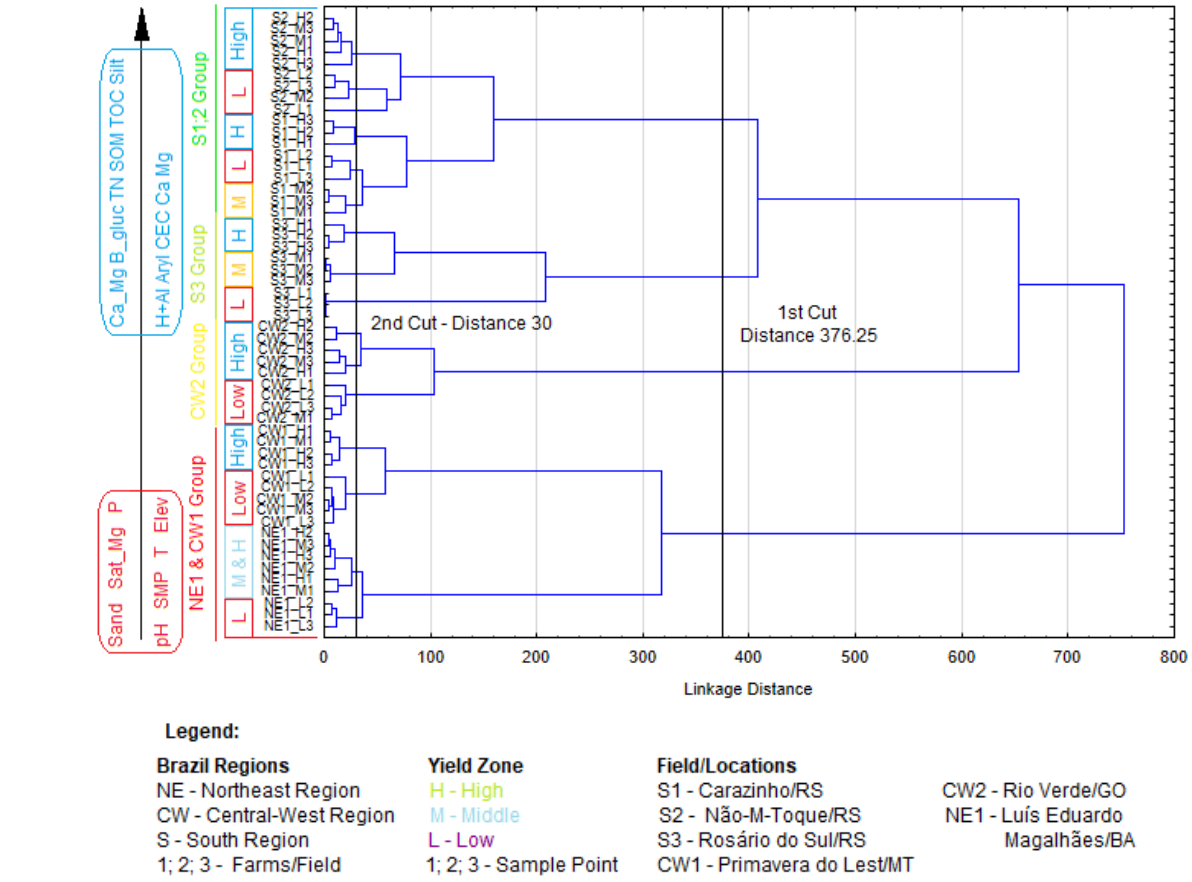
Mn^{2+} plays an important role in plant metabolic functions acting as an essential cofactor in the reduction of oxygen and stimulating the photosynthetic machinery by catalyzing the water division in photosynthesis [66]. In addition, Cu^{2+} is an essential component of various proteins that act in photosynthesis, respiration, and phytohormones linked to pollen production [64]. Therefore, the metallic micronutrients are essential to plant hormonal metabolism related to crop performance, as observed in the PCA analysis in our study (Figure 2). Their deficiency generally is associated with high pH (Figure 4), low SOM content, and dry soil [64], as verified in the Northeast field in our study (Table 2).

3.3. Enzyme Activity and Biodiversity under Varying Crop Yield Environments

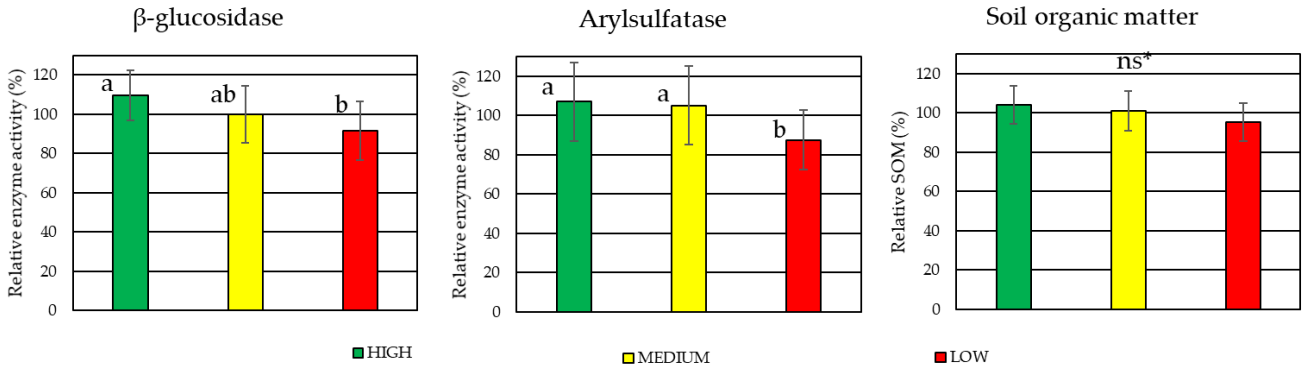
The cluster analysis shown in Figure 5a agrees with the cluster analysis shown in Figure 2a. In Figure 5a there is a division of data by agro-ecoregions and YEs. In the first cut the NE-1 and CW-1 fields were split from the others with a strong influence of sand content, Mg^{2+} saturation, P content, soil pH in water, temperature, and elevation. On the other hand, the CW-2 agro-ecoregion field was similar to the S-3 field, being in an intermediate position. Finally, the S-1 and S-2 (clay soils) fields were affected by β-glucosidase and arylsulfatase enzymes activity, Ca^{2+}/Mg^{2+} ratio, SOM content, TOC, CEC, Ca^{2+} and Mg^{2+} content, silt, and $H + Al^{3+}$, as previously discussed in the PCA analysis (Figure 2). In this way, four groups were constructed in Figure 5. The first group was associated with soil and weather detractor factors of enzyme activity, the second and third groups were in an intermediate position, and the fourth group was associated with promoter factors of enzyme activity.

In Figure 5a, the YEs within fields were distinguished, mainly in the second cut (Figure 5a). In Figure 5b, the different YEs, averaged across agro-ecoregions, were distinguished which highlighted that LYE was different from HYE and confirmed that the use of precision agriculture was an important tool for site-specific management in CA systems. Lorenz et al. [67] previously reported that β-glucosidase activity had a relationship with corn yield. In our study, the LYE had lower enzyme activity compared with HYE, with decreases of 18.0% and 19.6%, respectively (Figure 5b). These decreases were higher than

the difference in SOM content between these environments which was 8.6% but without significant statistical difference.



(**a**)



(**b**)

Figure 5. (**a**) Cluster analysis of case grouping among soil attributes, slope, climate and crop yield, $n = 54$; (**b**) β-glucosidase and arylsulfatase enzyme activity and SOM content relative to the average of the field in different yield potential environments (high, medium and low) in seven Brazilian fields from main agro-ecoregions. Values followed by the same letter do not differ statistically by Tukey test ($p < 0.05$), ns* = not significant, $n = 63$. Error bars correspond to standard deviation.

Lopes et al. [19] carried out a study aiming to establish critical levels of cellulase, β-glucosidase, arylsulfatase, and phosphatase enzyme activities for Central-West Brazilian Oxisols. They reported that high yielding soils generally had higher microbial biomass C, microbial respiration, and high enzymatic activity. These findings are in line with the results reported in our study (Figure 5b). Based on these critical enzyme activity levels [19], nearly

70% of the whole database of our study, comprising 63 sampling points, was classified as high enzyme level (>115 mg p-nitrophenol kg^{-1} soil h^{-1}) and 30% as moderate enzyme level (66–115 mg p-nitrophenol kg^{-1} soil h^{-1}). On the other hand, with arylsulfatase the enzyme activity levels were 55.5%, 30.2%, and 14.3% corresponding to high (>90 mg p-nitrophenol kg^{-1} soil h^{-1}), moderate (41–90 mg p-nitrophenol kg^{-1} soil h^{-1}), and low activity (≤40 mg p-nitrophenol kg^{-1} soil h^{-1}), respectively. It is important to highlight that these critical levels were proposed for the Central-West agro-ecoregions, and extrapolation to other Brazilian agro-ecoregions should be made with caution [15].

In one field (S-2), we investigated the relationship of the enzyme activity to soil DNA analysis (Figure 6). There was a linear relationship between β-glucosidase and arylsulfatase enzymes and the diversity of the microorganism community (fungus, bacteria, protist and archaea genera) with coefficients of determination of 0.85 ($p < 0.01$) and 0.79 ($p < 0.05$), respectively. These results reinforce the role of enzyme activity as an efficient bioindicator of soil health [15].

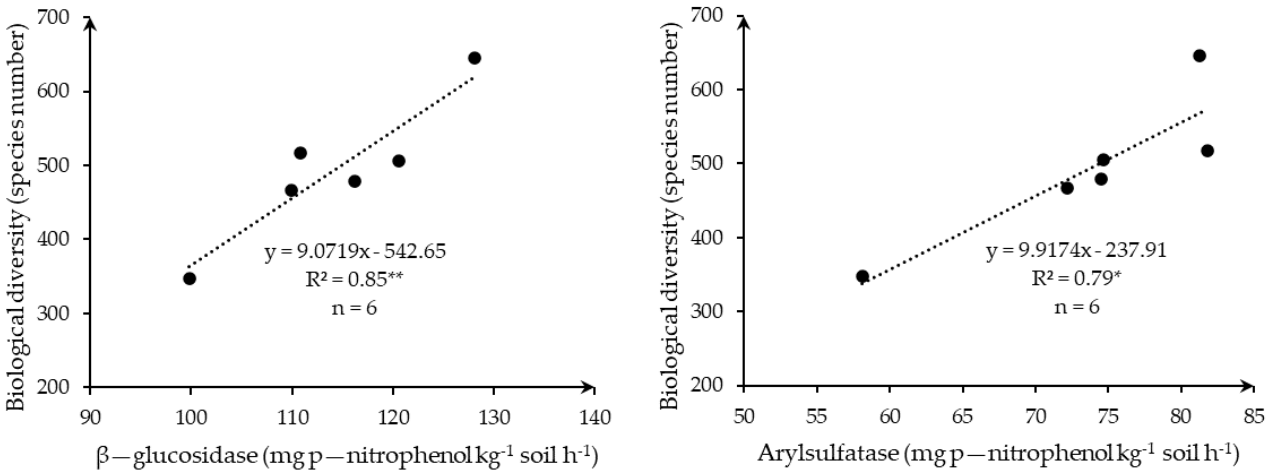


Figure 6. Relation of β-glucosidase and arylsulfatase enzymes activity under conservation agriculture and biodiversity assessed by DNA characterization in Southern Brazil field (S-2) Não-Me-Toque, RS. Significance codes: ** $p < 0.01$; * $p < 0.05$.

Bacteria and fungi are the main producers of extracellular enzymes that drive the process of degradation of complex polymeric compounds into simpler compounds (oligomers and monomers). Protists (protozoa and simple algae) play an important role in soil biogeochemical decomposition processes by bacteria that release nutrients to plants and create a "microbial loop" that is highly dependent on the quality of the crop residue input [68–70].

The microbiome species characterization in the S2 field (Figure 7a) showed that the HYE had a better balance among microorganism species, where the top 100 more abundant genera in the community comprised 52.9% of the total population. On the other hand, in the MYE and LYE the top 100 genera comprised 59.2% and 57.7% of the total microorganism community population suggesting lower microbial diversity [46].

The abundance of beneficial soil microorganism species is an important indicator of soil health. In our study, the genus *Mortierella* was the most abundant, accounting for 14.4%, 13.7% and 11.2% of total microbial population in HYE, MYE and LYE, respectively (Figure 7a). Ozimek and Hanaka [71], in a review of plant growth-promoting microorganisms, reported that some fungi, such as the *Mortierella* genus, are usually present in large amounts in the rhizosphere and help plant growth in hostile environments by increasing plant P uptake. Some microorganisms associated with legumes cover crops could provide phytohormones such as enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, responsible for the conversion of ACC (the precursor of ethylene in plants) into ammonia and α-ketobutyrate, which promotes plant growth by decreasing ethylene levels. This

bioenzyme could help plants to pest attack protection and increase their environmental adaptation skill [72].

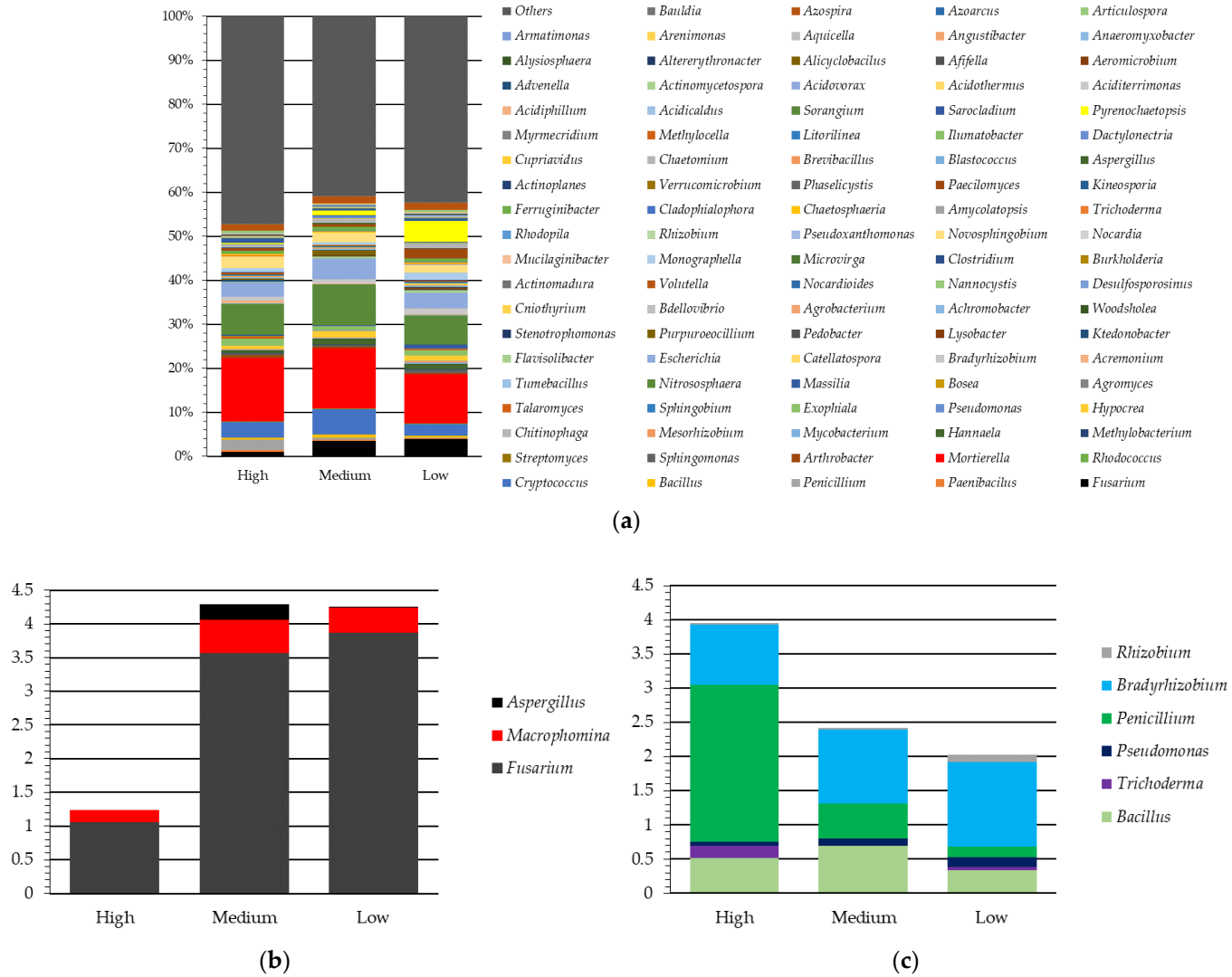


Figure 7. (**a**) Soil biodiversity evaluated by DNA barcode approach and accumulated proportion of most abundant genera of soil microorganisms classified as (**b**) pathogens and (**c**) beneficial to plant growth under varying yield environments under conservation agriculture in Southern Brazil (S-2 field) Não-Me-Toque, RS, $n = 6$.

Plant-microbe interactions in the rhizosphere are key factors in plant vigour and grain yield [73]. The main mechanisms involved are related to the bioavailability of nutrients, such as phosphate solubilization and biological N fixation, and biotic and abiotic stress alleviation [73]. Among the main genera of plant growth-promoting organisms are *Bacillus* and *Pseudomonas*, known as plant growth-promoting bacteria (PGPB) [14,73], and *Penicillium* and *Trichoderma*, known as plant growth-promoting fungus (PGPF), and soil biocontrol promoters [14,74–76]. Atmospheric N fixation could also be associated with the presence of *Bradyrhizobium* and *Rhizobium* [77]. These microorganisms were more abundant in HYE, being 64% and 96% more abundant than in MYE and LYE, respectively (Figure 7c). The organisms selected in our study were previously proposed by Shah et al. [5] in a review article regarding the role of soil microbes in sustainable crop production and soil health.

The most important plant pathogenic microorganisms found in our study were *Fusarium*, *Macrophomina*, and *Aspergillus* genera, that together were about 245% higher in MYE

and LYE than in HYE (Figure 7b). Therefore, the plants growing in MYE and LYE were under much higher pressure from soil plant diseases than in HYE.

Production fields managed under soybean monocropping and without diversification with cover crops are more prone to *Fusarium* root rot [78]. This is one of the most important soybean soil diseases that occurs in many fields in the North and South America [79,80]. Soybean lateral roots with *Fusarium* root rot may die and, in consequence, reduce plant nutrients uptake and exudates production that could support a more diversified soil biota [81]. It is important to highlight that this genus was the most prevalent in LYE and MYE and was associated with a decrease in plant-growth-promoting organisms (Figure 7c). Ranzi et al. [80], evaluating the soybean and maize in monocropping systems, were able to identify up to nine *Fusarium* species connected with the lack of crop diversity and high soil compaction.

The genera *Penicillium*, *Bacillus*, *Pseudomonas*, and *Trichoderma* play an important role in plant protection from *Fusarium* attack [74–76,82–84] through the production of antibiotic bioproducts, the modulation of ACC deaminase expression, and by increasing soil nutrient uptake that results in higher plant photosynthesis rates and plant vigour status. In addition, these microorganisms could induce a reprogramming of plant gene expressions that increases plants' ability to cope with biotic and abiotic stresses [74,75,85–87]. In our study, *Penicillium* was strongly associated with YEs being 351% and 1338% higher in HYE than in MYE and LYE, respectively (Figure 7). Therefore, in MYE and LYE, the lower population of *Penicillium* was linked with a higher population of *Fusarium* (Figure 7).

In our study, soil compaction was evaluated based on the soil penetration resistance (PR) in the three YEs investigated (Figure 8). Our results are consistent with those of Pott et al. [23] in the same field of our study that reported higher PR, lower macroporosity and lower water infiltration in LYE than HYE. In our study, PR was higher at depths greater than 0.15 m and followed the decreasing order: LYE > MYE > HYE (Figure 8). PR > 2.5 MPa is assumed to be a critical value for soybean root growth and crop yield [88]. LYE had PR greater than this critical value, while MYE and HYE did not reach this reference at any soil depth (Figure 8). High PR values could affect soil aeration, mainly in clay soils, and stimulate the occurrence of *Fusarium* under frequent rainfall conditions [89–91].

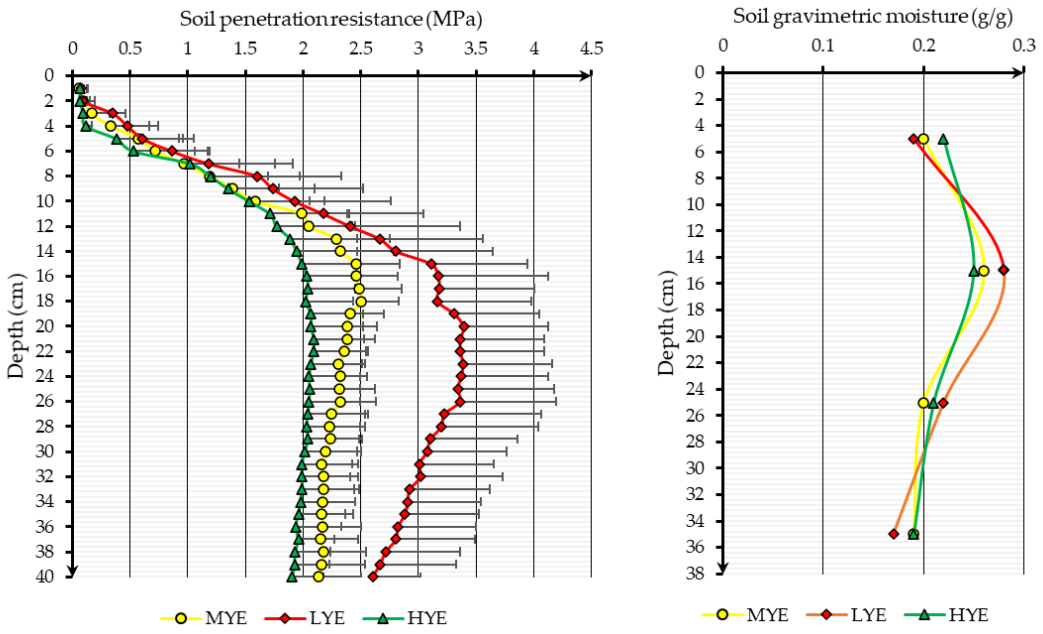
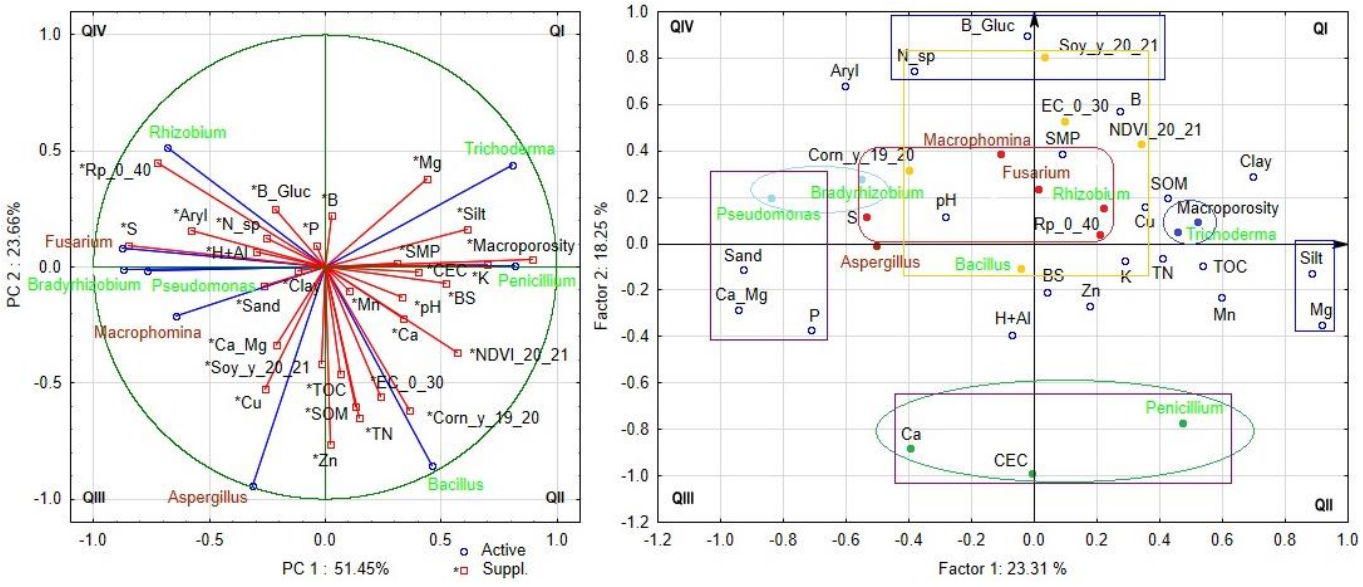


Figure 8. Soil penetration resistance and soil moisture in varying environment yields in conservation agriculture in South Brazil (S-2 field). Error bars correspond to standard deviation.

Figure 9a shows the PCA that explains 75.1% of the variance in the data of plant-growth organisms and plant pathogenic organisms and their relationships with soil attributes. This information is important for biologically oriented soil management. There was a positive effect of Ca^{2+}, Mg^{2+}, K^{+} content, corn yield (season 2019/20), macroporosity, silt, SOM, TOC, TN, CEC, EC, pH SMP, Zn^{2+} and soil pH in water, with *Trichoderma*, *Penicillium* and *Bacillus* positioned in QI and QII (Figure 9a). On the other hand, the pathogenic organisms represented by *Fusarium*, *Macrophomina* and *Aspergillus* were associated with PR, S, Cu^{2+}, Ca^{2+}/Mg^{2+} ratio, sandy content, H + Al^{3+}, soybean yield (2020/21), were positioned in QIII and QIV (Figure 9a).

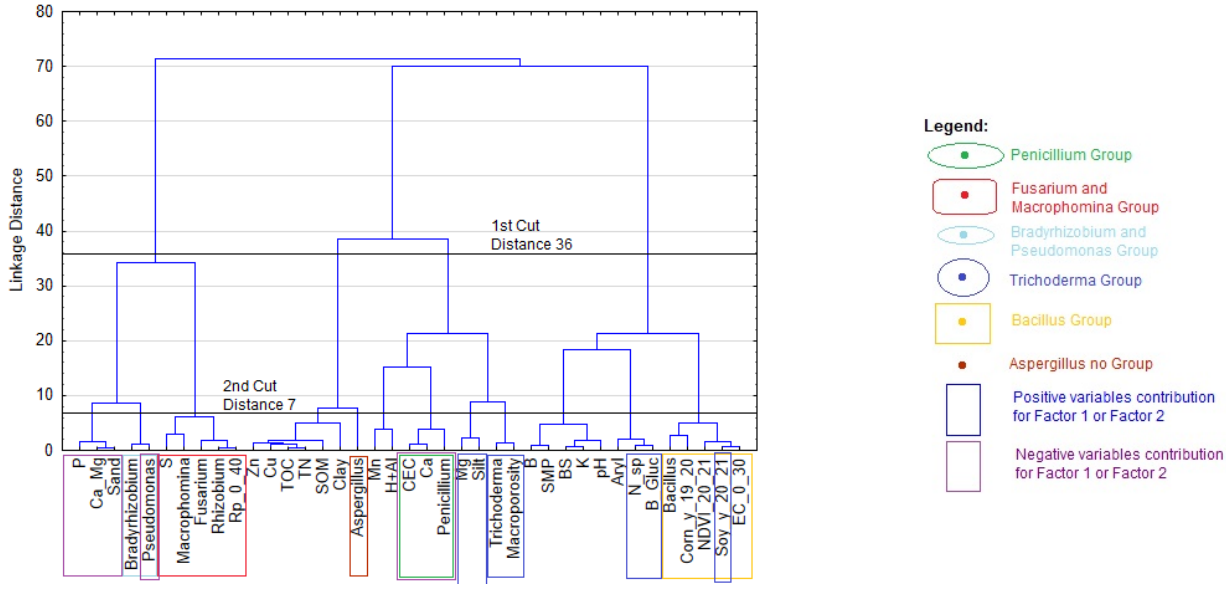
There was an antagonistic relationship between *Penicillium* (QI) and *Fusarium* (QIV), and between *Trichoderma* and *Macrophomina* (Figure 9a). These results could be explained by the biocontrol effect *of Penicillium* and *Trichoderma* The *Bacillus* was in the same quadrant (QII) of TN, SOM and TOC soil attributes.

In the factorial analysis, it was shown that: *Penicillium* was associated with Ca^{2+} content and CEC (Figure 9b,c); *Bradyrizobim* was associated with *Pseudomonas* (Figure 9b); *Trichoderma* was associated with macroporosity (Figure 9b,c); and *Bacillus* was associated with EC, biodiversity, crop yield, and NDVI (Figure 9b,c). On the other hand, *Macrophomina*, *Fusarium* and *Aspergillus* were associated with high PR, an indicator of soil compaction. In addition, the imbalance in lime and fertilization expressed by high soil pH in water and high P content concentrate in a shallow layer affected these plant pathogenic organisms. These results reinforce the notion that soil attributes are an important driver of soil biota and that they could be managed at the farming level to support a diversity of organisms that are plant-growth promoters. However, further studies are required to more fully understand these relationships.



(**a**) (**b**)

Figure 9. *Cont.*



(**c**)

Figure 9. (**a**) Projection of the dispersion of variables by principal components analysis (PCA), among most abundant genera of soil microorganisms classified as pathogens and beneficial to plant growth evaluated by molecular analysis of DNA, soil attributes and crop yield growth in varying yield environments under conservation agriculture in Southern Brazil (S-2 field) Não-Me-Toque, RS. Proportion of genera *Penicillium*, *Bacillus*, *Pseudomonas*, *Trichoderma*, *Bradyrhizobium*, *Rhizobium*, *Fusarium*, *Macrophomina* and *Aspergillus* in the soil microbiome were taken as the main variables in the analysis. The supplementary variables (*) were: soybean crop grain yield of the 2020/21 season (Soy_y_20_21); corn crop grain yield of the 2019/20 season (Corn_y_19_20); normalized difference vegetation index (NDVI_20_21), species number in the soil by molecular analysis (N_sp); β-glucosidase (B_Gluc) and arylsulfatase (Aryl) soil enzyme activity; sand; silt; clay; total nitrogen (TN); total organic carbon (TOC); potential of hydrogen (pH); pH SMP index (SMP); phosphorus (P); potassium (K); soil organic matter (SOM); Ca^{2+}; Mg^{2+}; cation exchange capacity (CEC); potential acidity (H+Al); bases saturation (BS); sulphur (S); Zn^{2+}; Cu^{2+}; boron (B); Mn^{2+}; calcium/magnesium relation (Ca_Mg); soil electrical conductivity of 0–0.30 m depth (EC_0_30); soil penetration resistance, average of 0–0.40 m depth (PR_0_40), and macroporosity of 0.05–0.10 m; (**b**) dispersion of variables and (**c**) cluster analysis according to their contribution to the formation of Factors 1 and 2; $n = 6$.

4. Conclusions

The β-glucosidase and arylsulfatase enzyme activity were efficient indicators of soil biodiversity under CA. In addition, the activity of these enzymes serves as an efficient tool to distinguish low yield environments from high yield environments within fields.

In general, fine soil particles (clay and silt), high CEC, high Ca^{2+} content, high Ca^{2+}/Mg^{2+} ratio, high TOC, TN and SOM were promoters of β-glucosidase and arylsulfatase soil enzyme activity in the fields of the main Brazilian agro-ecoregions. On the other hand, soil pH in water (>6.5), high sand content, high P content concentrated in a shallow layer, high temperature, low Cu^{2+} and Mn^{2+} availability decreased the activity of these enzymes.

A large proportion of data points investigated (40%) had low SOM, TOC and TN content, creating conditions that were associated with low enzyme activity and restricted biodiversity. These results reinforce the view that the application of the three interlinked principles of CA operate synergistically to build and sustain soil health in production systems. In addition, imbalance in soil correction and fertilization input affects soil enzyme activity. The overuse of these inputs could result in high soil pH in water, high phosphorus concentration in specific soil depth, low copper and manganese availability, narrow Ca^{2+}/Mg^{2+} ratio, and high Mg^{2+} saturation that together with sandy texture and high temperature were associated with low enzyme activity. On the other hand, low or lack of use of these inputs could result in low Ca^{2+} and Mg^{2+} content, low soil pH in water, high

Al^{3+} content, low base saturation and CEC that were associated with low SOM content and low TOC and TN that were also linked to low enzyme activity.

Through the soil microbiome characterization, it was possible to add a new data layer that, together with data on soil and plant attributes, helped to explain the varying yield environments within a production field. In our study, high yield environments had a more diverse soil microbial community with a higher presence of biota that promote plant growth (*Bacillus*, *Penicillium*, *Trichoderma*, *Pseudomonas*, *Bradyrhizobium* and *Rhizobium*). In the low yield environments, there was a higher presence of *Fusarium* and *Macrophomina* that were negatives for plant growth. These pathogenic organisms were associated with the presence of high soil penetration resistance and low microbiota diversity as a consequence of soil compaction.

It is concluded that applying the three integrated principles of CA with a focus on crop rotation and cover crops in the cropping system results in enhancement of soil health and crop productivity. The key drivers in this soil health regeneration process are the restoration of soil organic matter and total nitrogen content through crop diversification, calibrated correction of plant nutrients with fertilization that focuses on increased Ca^{2+} content, avoidance of soil compaction and stimulation of plant root growth that will support plant-growth promoting microorganisms and a diverse soil biota community.

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Conflicts of Interest: The authors declare no conflict of interest.

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