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Soil health checkup of Brazilian Conservation Agriculture farming systems

J.H. Passinato[1], T.J.C. Amado[1], J.A.A. Acosta[2], A. Kassam[3]
1.Department of Soils, Rural Sciences Center /Santa Maria Federal University, Santa Maria, RS, Brazil.
2 Drakkar Solos Consultoria/Santa Maria, RS, Brazil.
3 School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR, UK.

Corresponding author: *proftelmoamado@gmail.com.*

ABSTRACT

Brazil has around 35 M ha of cropland managed under Conservation Agriculture (CA) grain farming placing the country as one of the world's largest area. CA has many advantages in relation to intensive tillage-based farming by providing soil erosion control, organic matter restoration, saves labor, time and fuel and offers competitive yields. Documented effect of CA on soil health at the farm level is still relatively scarce. This study was carried out aiming to investigate the enzyme activity analysis as an indicator of CA soil health in main Brazilian agro-ecoregions. For that, seven fields located in main grain producing regions in South, Central-West and Northeast were selected. In each of them three environments (high, medium and low yield) were defined based on crop yield records and satellite images. The chemical soil analysis (SOM, P, K, Ca, Mg, S, Al, B, Cu, Zn, BS, CEC, pH) and physical analysis (soil texture, electrical conductivity - ECa) were performed. The activity of soil enzymes β-glucosidase and arylsulfatase was evaluated in 63 sampling points spread in four States. These enzyme activities have been recently proposed as key indicators of Brazilian soil health. One field with larger data base was selected for DNA characterization in order to more deeply understand soil health and its relationship with field crop yields. The results show that β-glucosidase and arylsulfatase activities have positive relationships with SOM, clay, silt, Ca content and CEC. Also, these enzyme activities had negative relationship with sand texture. The enzymes were sensitive to soil productive capacity within field. Tropical Brazilian soils usually are acid, with low activity clay, and dystrophic character. As a consequence, soil acidity correction, SOM restoration and soil fertility and CEC increase were important strategies to improve biological activity. In the study, SOM contents higher than 3.5% were associated with high β-glucosidase and arylsulfatase enzyme activities. However, around 37% of the data points had low SOM that were associate with low enzymes activity. The enzymes were also efficient indicators of soil biodiversity assessed by DNA characterization. Finally, the study concludes that following the three integrate principles of CA with focus on crop rotation and cover crop use, SOM restoration, alleviation of soil acidity, and increase in Ca content were key drivers in the restoration of soil health, with positive consequence for crop yield.

Keywords: *Agro-ecoregions, Soil Enzymes, Soil DNA, Soybean Yield, Soil Organic Matter.*

1. INTRODUCTION

The projected global population growth over the next decades will increase the demand for food, fiber, biofuel, energy, water and other agricultural products. As a consequence, there will be growing pressure on natural ecosystems and agroecosystems, which are already facing sustainability challenges due climate change, soil degradation and loss of biodiversity, compromising their environmental services at different scales (Kassam et al., 2009). This scenario highlights an imperative need for the development of more sustainable agricultural systems. Therefore, business-as-usual attitude towards agricultural production in most world regions will fail to deliver sustainable production intensification to meet future needs (Shaxson, 2006; Kassam et al., 2009). Therefore, there is an urgent need of redesign agriculture production systems in order to decrease environmental, economic and social costs associate with current intensive tillage and chemical-based cropping systems.

Conservation Agriculture (CA) has been practiced for more than four decades in the pioneers regions in North and South America and based on the positive results obtained, it has been gradually spreading worldwide in filling the important gaps of business-as-usual agriculture in addressing societal challenges. The three principles that define CA are: a) minimizing soil disturbance by mechanical tillage avoiding inversion of soil layers, breakdown and mixing of crop residues into the soil, and minimizing fast residue decomposition and aggregate disruption; b) maintaining year-round diverse organic matter cover with living and dead plant material over the soil; and c) diversifying crop rotations and associations, enhancing a consortium of cover crops to fill up all spare time windows between main cash crops, including nitrogen fixing legumes and soil return of high quality crop biomass (Kassam et al., 2009; 2018; Leal et al., 2020). Currently, Brazil has about 35 M ha under CA cropland spread in different agro-ecoregions, with varying levels of CA implementation due to the continental dimensions of the country (fifth largest t country in the world in terms of area). As a consequence, there is a complex interaction of weather, soil and production management including during the early years of transition into CA that may have consequences for soil health that may not be well understood.

Soil health can be defined as the capacity of a specific soil type to function, with natural or managed boundaries, in order to sustain plant and animal productivity capacity, maintain/enhance water and air quality, support human health and biological diversity (Doran and Zeiss, 2000; Doran and Parkin, 1994; Garbisu et al., 2011). Moreover, ‘conservation-effectiveness’ encompasses not only conserving soil and water, but also enhance the soil biotic component that is the basis of sustainability (Kassam et al., 2009). In an analogous way, the ‘crop production-effectiveness’ encompasses not only the maintenance of soil chemical nutrient levels above some critical levels but provide friendly habitat to diverse microbiome that will stimulate nutrient cycling and enhance root uptake of 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 (Kremer, 2017). In addition, soil health can be understood as a subcomponent of a bigger ecosystem health. A healthy ecosystem relies on efficient nutrient cycling, high photosynthesis rate, energy flow, stability and resilience to stress (Van Bruggen et al., 2006; Tripathi et al., 2020). In this sense, there is a solid linkage between ecosystem health and soil heath expressed by microbial activity, biodiversity and community stability (Tripathi et al., 2020). Therefore, building soil health through farming practices is one pathway for ensuring sustainable agriculture. The microbiome living in the rhizosphere is a hot-spot because the microbiota act as plant growth-promoters and plant growth-regulators, affecting root growth with positive effects on plant nutrients uptake, water use efficiency and environmental adaptation (Khan et al., 2020; Mendes et al., 2018).

Building a diverse microbiome in the rhizosphere is also needed to suppress or alleviate pressures from plant pathogens, decreasing disease incidence and severity resulting in more vigorous plants that are more resilient to stress (Van Bruggen et al., 1996; Toor and Adnan, 2020; Tripathi et al., 2020).

The soil physical attributes, particularly soil texture, structure, compaction, bulk density, aggregation, porosity and water availability, and chemical attributes, especially pH, SOM, nitrogen, plant exudates, salinity, aluminum, hydrogen, CEC and nutrients interact with cropping system and weather conditions, driving the microbial activities and their functional diversity (Tripathi et al., 2020). The microbial activity and diversity are sensitive bioindicators of soil management quality (Mendes et al., 2018; Leal et al., 2020). Therefore, assessing the soil microbiome and enzymes activity may provide early insights about the quality of soil management and forecasting if it is improving the soil or promoting degradation before advance stages are reached (Tripathi et al., 2020).

The main objective of this study was to assess soil health through enzyme activity of long-term CA croplands in main Brazilian agro-ecoregions. Moreover, in one select field the DNA characterization was investigated in order to capture microbiome diversity in different crop yield environments within the field.

2. METODOLOGY

2.1. Agro-ecoregions, croplands and within-field yield environments

This study was carried out in seven grain fields managed during long-term under CA that are located in the main Brazilian agro-ecoregions: South, Central-West ('Cerrado') and Northeast (Fig. 1 and Table 1). In each one three within-field yield environments (high, medium and low yield) were delineated based on crop yield maps and satellite images (NDVI) according to the available data. The high yield environment was classified as > 110% average crop yield, medium as 80 – 110% and low as < 80%.

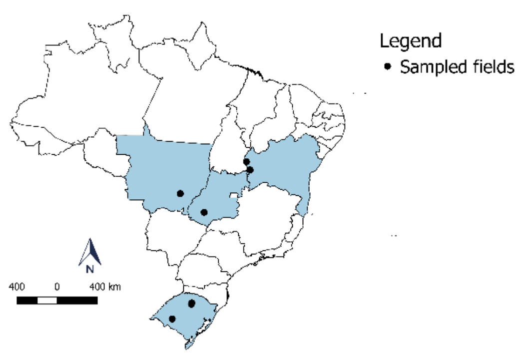

Fig. 1 Geographical distribution of the fields sampled in the main agro-ecoregions of Brazil.

Table 1: Fields locations, areas (ha), average annual temperature (T) (°C), annual accumulated precipitation (P) (mm), average altitude (E) (m) and soil texture.

Field	Localization	Area	T	P	E	Soil texture
		ha	°C	mm y^{-1}	m	
S-1	Carazinho – RS	60.1	18.3	1483	565	Clay loam
S-2	Não-Me-Toque – RS	124.0	19.0	1771	500	Clay loam

S-3	Rosário do Sul – RS	25.0	19.5	1493	155	Sandy loam
CW-1	Primavera do Leste – MT	348.8	24.0	1471	650	Sandy clay loam
CW-2	Rio Verde – GO	509.8	23.1	1294	875	Clay loam
NE-1	Luís Eduardo Magalhães – BA	1376.1	23.6	881	830	Sandy clay loam
NE-2	Placas – BA	690.9	25.0	1089	880	Sandy clay loam

*Soil texture classified according to Soil Survey Staff (2014); 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 to enzyme analysis, DNA characterization and crop yield

In each yield environment of the seven fields, soil at 0-0.10 m depth was collected with three repetitions for chemical and enzyme activity analyses totaling 63 georeferenced sampling points. Soil samples for enzyme activity analysis were collected 40 days after crop emergence using manual shovel. Seven sampling points comprised one in the center of the 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 Mendes et al. (2019) methodology. The β-glucosidase e arylsulfatase enzymes activity analysis followed Tabatabai (1994) methodology.

The chemical analyses were soil water pH (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 colorimetrically, using molybdenum blue (Embrapa, 2011). Calcium (Ca), magnesium (Mg) and aluminum (Al) were extracted using 1.0 mol L−1 KCl solution. Ca and Mg were determined by atomic absorption spectrophotometry. Al was titrated with NaOH 0.025 mol L^{-1}. The cation exchange capacity (CEC) pH 7 was determined by the sum of the exchangeable bases (K, Ca, and Mg) plus Al+H according to Tedesco et al. (1995). The soil texture was determined by pipette method according Teixeira et al. (2017).

One of the fields of the Aquarius project, that had a large available data base, was used to do DNA characterization. The soil is clayey, kaolinitic and classified as a Rhodic Hapludox (Soil Survey Staff, 2014). The cropland has been managed under CA since 2002 and more details can be found in Pott et al. (2019). In the growing season of 2019/2020 soil samples were collect at 0-0.10 m and sent to Biome Markers® (https://biomemakers.com) in United States for molecular analysis of the microbiota. DNA extraction was performed with the DNeasy 420 PowerLyzer PowerSoil Kit from Qiagen (Imam et al., 2021). To characterize both bacterial and fungal microbial 421 communities associated with bulk soils and rhizosphere samples, the 16S rRNA and ITS marker 422 regions were selected. Libraries were prepared following the two-step PCR Illumina protocol 423 using custom primers amplifying the 16S rRNA V4 region and the ITS1 region described 424 previously (Imam et al., 2021). DNA sequencing was conducted in an Illumina MiSeq instrument using pair-end 425 sequencing (2x300bp). The platform BeCrop® was used in the study, and more details can be found in Imam et al. (2021).

2.3. Statistical Analysis

The results of enzyme activity, chemical analyses and crop yields were submitted to variance analysis ($p<0.01$ e $p<0.05$) and Pearson’s correlation. The relationship of SOM and number of species and enzyme activity were analyzed by linear and quadratic adjustments. The enzyme activity and SOM relative average in each within-field yield environment were compared based on the Tukey test ($p<0.05$).

3. RESULTS

3.1. Soil attributes and relationship with soil enzymes activity

According to agro-ecoregion, the soil attributes show differences in their effect on soil enzymes activity (Table 2). Soil texture had an effect on soil enzyme activity in the South and Central-West regions but not in the Northeast. In general, in the South and Central-West regions the increase of sand content was associated with a decrease in enzymes activity. On the other hand, in Northeast where the soils are very sandy and there is a narrow variation in soil texture, this relationship was not verified. Soil texture had influence on structure, CEC, SOM content, soil temperature and water holding capacity. Typically clay soils are expected to have higher microbial biomass and enzyme activity than sandy soils under similar conditions. Ji et al. (2014) reported that the number of soil actinomycetes and fungi in clay soil was 151% and 43% higher than in loam soil. The authors linked this result to fine clay particles that hold water and SOM. Elliot et al. (1980) and Alvarez et al. (2002) highlight the protective effect of clay to microbiome. In our study the clay content had relationship with β-glucosidase in South, and with arylsulfatase in South and Central-West regions (Table 2).

Table 2. Pearson's correlation of β-glucosidase and arylsulfatase with SOM, soil texture, CEC and Ca content. * significant $p<0.05$; ** significant $p<0.01$; ns= not significant; n=63

	Region	SOM	Sand	Silt	Clay	CEC	Ca
β-glucosidase	South	0.78*	-0.61*	0.39**	0.48**	0.49**	0.35ns
	Central-West	0.83*	-0.91*	0.85*	0.43ns	0.58**	0.56**
	Northeast	0.67*	-0.07ns	0.24ns	-0.13ns	0.31ns	0.20ns
	Brazil	**0.77***	**-0.76***	**0.70***	**0.41***	**0.67***	**0.59***
Arylsulfatase	South	0.79*	-0.72*	0.35ns	0.67*	0.55*	0.38ns
	Central-West	0.80*	-0.89*	0.82*	0.47**	0.51**	0.53**
	Northeast	-0.13ns	-0.08ns	-0.06ns	0.18ns	-0.14ns	-0.18ns
	Brazil	**0.65***	**-0.64***	**0.49***	**0.53***	**0.82***	**0.72***
SOM	South	-	-0.78*	0.24ns	0.84*	0.37ns	0.13ns
	Central-West	-	-0.83*	0.81*	0.26ns	0.72*	0.75*
	Northeast	-	-0.29ns	0.30ns	0.13ns	0.54**	0.38ns
	Brazil	**-**	**-0.86***	**0.78***	**0.49***	**0.61***	**0.46***

The CEC had a positive effect on enzymes activity in the South and Central-West regions. In tropical soils, the CEC is dependent on clay mineralogy and content and SOM. Soares et al. (2005) and Bayer et al. (2000) reported that Oxisols, which are highly weathered, had around 80% of its CEC associated with SOM. The interaction between SOM and clay minerals (organomineral complex) increases soil aggregation and physically protects SOM from microbial degradation. Ferreira et al. (2018) and Xu et al. (2014) reported that CEC and base saturation were drivers of SOM gain in tropical CA soils. These results indicate that nutrient management plays an important role in SOM recovery in dystrophic tropical soils.

In this study, the Ca content had positive relationship with enzyme activity in the Central-West region. In addition, country averaged Ca had relationships of 0.59 and 0.72 with β -glucosidase and arylsulfatase enzyme activity, respectively (Table 2). Previously, Pires et al. (2020) reported that Ca was a driver of β-glucosidase in a South CA long-term experiment. Ca serves as a constituent of cell walls and membranes and can act as a physical barrier against pathogens (Thor, 2019). In addition, Ca increases root growth, mainly of the fine roots that are very active in providing exudates to microbial rhizosphere community. Finally, Ca is important for soil aggregation and carbon stabilization under CA (Ferreira et al., 2018).

The SOM had stronger relationship with enzymes activity in the South and Central-West regions with r values of 0.67 to 0.83, respectively. In the Northeast region the SOM had a relationship with β-glucosidase but not with arylsulfatase. Moreover, in this region the only soil attribute that had a relationship with enzyme activity (β-glucosidase) was SOM. In the Fig. 2 it is shown that SOM had a linear positive relationship with β-glucosidase which explained around 60% of variability of this enzyme activity. The maximum enzyme activity was reached with maximum high SOM content (>5%). The arylsulfatase had a quadratic relationship with SOM, with maximum activity reached at 3.55 %. Xu et al. (2014) reported that SOM had a positive relationship of 0.83 with enzyme activity and N content. The authors explained that microbes need nutrients coming from labile fractions of SOM that they use as energy and nutrient sources. In addition, SOM retains soil moisture, enhances CEC and aggregation that had enhanced microbial biomass and enzyme activity. A recent exploratory study of soil analyses from South Brazil laboratories (n=35,362) reported that 55% of the total had SOM <2.5% (Tiecher et al, 2016). In our study, we had around 40% of the data points with low SOM (<2.5%) that were associated with low enzymes activity (Fig. 2). These data suggest an urgent need to revise the use of cropping system enhancing rotations and cover crops in order to build up soil health in this important parcel of Brazilian CA regions.

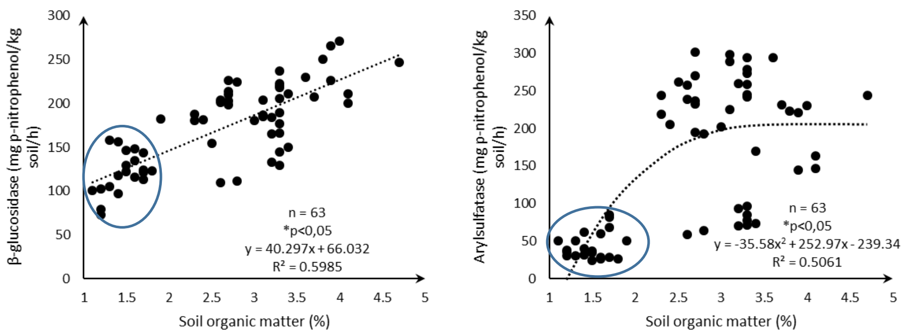

Fig. 2 Correlations between the activity of β-glucosidase and arylsulfatase enzymes and soil organic matter (SOM). * Correlations above are considered significant.

The SOM restoration and enzyme activity are strongly linked with CA principles. Pires et al. (2020) reported that long-term adoption of CA (32 years) increased SOM at the soil surface compared to intensively tilled soils. Moreover, the introduction of crop diversification increased SOM protection and aggregate stability enhancing the soil microbial diversity and enzyme activity. In their study, the β-glucosidase activity was 69% higher in CA than in tillage-based systems. Moreover, β-glucosidase increased by 23% under CA with crop rotation compared to no-till monocropping systems. The biological improvement associated with crop diversification under CA was fully offset by mechanical soil tillage. Soil disturbance avoidance stimulates growth of fungi hyphal networks, which allows fungi to establish bridges at the mulch-soil interface facilitating SOM stabilization.

3.2. Enzyme activity and biodiversity in varying crop yield environments

The β-glucosidase and arylsulfatase enzyme activity were efficient in distinguish high and medium yield environments from the low yield definied base on previous crop yield records and satellital images (Fig. 3). Accordingly, Lorenz et al. (2020) reported that β-glucosidase had a relationship with corn yield but arylsulfatase did not show a relationship with soybean yield.

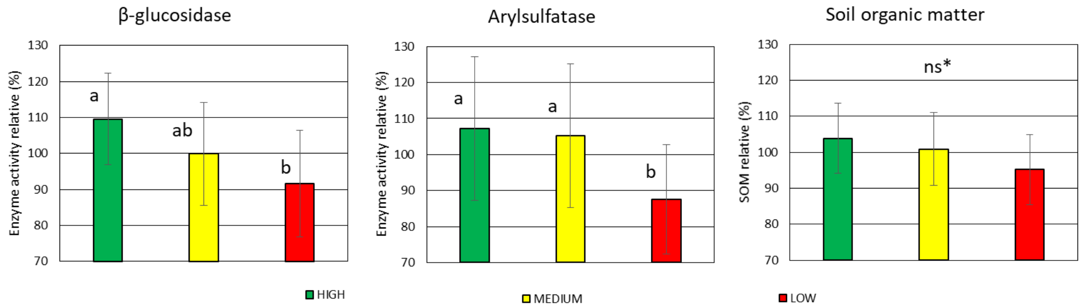

Fig. 3 Relation of β-glucosidase and arylsulfatase enzyme activity under Conservation Agriculture with varying yield environments in main Brazilian agroecosystem regions.

The β-glusosidase and arylsulfatase had a positive linear relationship with the biodiversity assessed by DNA characterization (Fig. 4). The coefficient of determination between β-glucosidase and arylsulfatase with the number of microbiome species were 0.85 and 0.79, respectively. These results support the enzyme activity level to be a sensitive indicator of soil health (Mendes et al., 2018).

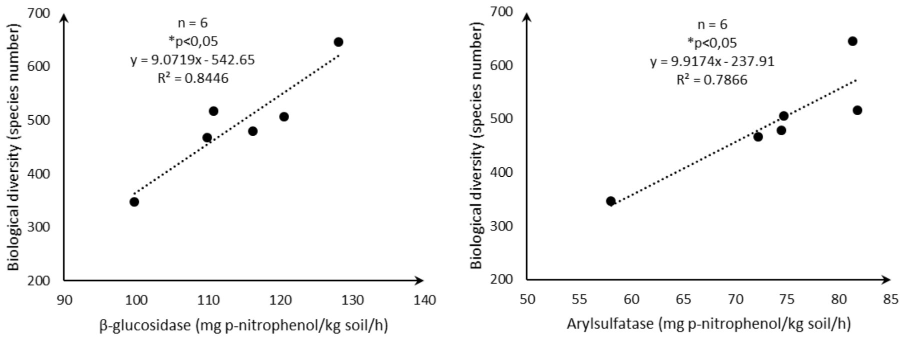

Fig. 4 Relation of β-glucosidase and arylsulfatase enzymes activity under conservation agriculture and biodiversity assessed by DNA characterization. * $p<0.05$.

4. CONCLUSIONS

In general, the fine soil particles (clay and silt), CEC, calcium content and SOM had a positive relationship with β-glucosidase and arylsulfatase activity in the Brazilian agro-ecoregion investigated.

The β-glucosidase and arylsulfatase enzymes activity were efficient indicators of biodiversity under Conservation Agriculture. Also, the enzyme activity was an efficient tool to distinguish the variation between within-field yield environments.

A large proportion of data points investigated (40%) had low SOM content that causes low enzymes activity and restricts biodiversity. These results reinforce the conclusion that the three principles of Conservation Agriculture operate synergistically in order to build up soil health in production systems.

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