Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-021-00606-z

ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Enzyme activities in a sandy soil of Western Bahia under cotton production systems: short-term effects, temporal variability, and the FERTBIO sample concept

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Received: 20 May 2021 / Accepted: 30 August 2021

Abstract
Enzyme activities (EAs) and the FERTBIO sample concept have been increasingly adopted as a novel approach to estimate the soil quality in Brazil. However, the performance of this strategy in sandy soils of the Cerrado biome remains unclear. During 2 years, in a Cerrado's sandy soil, the short-term effects of ten different cropping systems (conventional tillage or no-tillage associated with monoculture, rotations, and/or successions) on the activities of β-glucosidase, acid phosphatase, and arylsulfatase were studied. Issues related to annual variability and the feasibility of using the FERTBIO sample concept for soil enzymes activities were also evaluated. Soil samples were collected at three different depths (0–10 cm, 10–20 cm, and 20–40 cm) in March 2017 and February 2018. Five years since the beginning of the experiment, the presence of cover crops and no-till promoted improvements in EAs evidencing the importance of regenerative management practices for the sustainability of agroecosystems in sandy soils. Regardless of the cropping systems and depths evaluated, soil organic carbon and EAs showed low temporal variation during the 2 years of monitoring. Our results also showed that it is possible to use the FERTBIO sample concept for the Quartzipsament soils of Western Bahia, Brazil.

Keywords β-glucosidase · Acid phosphatase · Arylsulfatase · Soil health · Soil quality · Cerrado

Introduction

Cotton is one of the main Brazilian commodities. In 2020/2021, about 1.5 million ha was cultivated with this crop, resulting in a production of approximately 4 million tons [1]. Advanced high technology and good management practices have made Brazil the world's fourth largest producer and second largest exporter of cotton lint. Western Bahia (WB) is one of the prominent regions in national production, accumulating almost all production in the state of Bahia, which is the second largest producer in Brazil, just behind the state of Mato Grosso [1]. Located in the transition between two important Brazilian biomes (the Cerrado and Caatinga), WB stands out for its intense agricultural activity, with 5.5 million ha of arable land, of which 2.2 million was cultivated with soybean, maize, and cotton in 2018/2019 [2]. In this region, it is common to grow grain crops in light-textured soils, susceptible to erosion and groundwater contamination due to low cohesion between soil particles and poor aggregate stability [3–5].

Cotton monoculture, followed by a winter fallow, in association with conventional tillage (CT) practices, is still used in this region. This non-conservationist management system favors soil erosion [6, 7], reducing the already low organic matter content of these sandy soils [8, 9]. However, in recent years, the monoculture of this crop has been decreasing due to rotation with soybean and corn [2]. More recently, the integration of deep-rooted *Brachiaria* grasses (*Urochloa* spp.) either intercropped with maize, or succeeding soybean, is also an option that has been successfully adopted. The presence of *Brachiaria* either as living cover-crop or pasture for the cattle during the

Responsible Editor: Acacio Aparecido Navarrete

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Published online: 18 September 2021

dry/winter season (a cropping system known as integrated crop-livestock, ICL) increases the input of plant residues and provides soil protection during the dry season, favoring a more biologically active edaphic environment [10].

These and other crop diversification and integration strategies need to be evaluated in order to establish sustainable and cost-effective production systems in these stress-prone environments [11]. Overall, a major challenge in the Cerrado's production systems is to increase the availability of plant biomass during the dry season in order to provide feed to animals and/or to protect soil by forming straw for the no-tillage systems (NT). The adoption of NT in conjunction with intensive cropping systems with high plant biomass input is capable of restoring soil organic carbon (SOC) content in the sandy soils of WB [9]. This challenge is particularly important in these soils because they are inherently low in soil organic matter (SOM) and prone to wind erosion. Temperature increases and irregular rainfalls during the rainy season also impose adjustments in the current cotton production systems of WB, in order to increase their resilience to abiotic factors.

Microbial communities are the main source of enzymes in soils, which are key in SOM decomposition and in C, N, P, and S cycling in soil [12–14]. Already observed for temperate conditions, soil enzyme measurements also have great potential as soil quality indicators in the tropics due to their sensitivity, responding faster to shifts in management practices than parameters like soil organic matter [10, 15]. Other advantages include their close relationship to several aspects of soil functioning, precision, coherence, simple, and inexpensive analytical determinations [16, 17]. Based on their close association with crop yields and SOM, interpretation tables for soil enzymes activities (EAs) were developed [18–20], establishing reference values (low, moderate, and adequate) for these bioindicators in tropical soils. More recently, reference values for EAs in clayey tropical soils were reported even with samples collected at the post-harvest stage and air-dried under room temperature, the so-called FERTBIO soil sample concept [10].

In sandy soils of tropical environments, there is little information regarding the effects of tillage practices and cotton-cropping systems (monocrop, succession, and rotation) on EAs. Thus, the present study aimed to evaluate the short-term effects of different cropping systems (CT and NT associated with monoculture, rotations, and/or successions) on EAs. The study was conducted with soil samples collected at three different depths (0–10 cm, 10–20 cm, and 20–40 cm) for a period of 2 years. In addition, issues related to annual variability and the feasibility of using the FERTBIO sample concept [10] for enzymatic activity analysis in sandy soils of the Cerrado region were also evaluated.

Materials and methods

Location and characterization of the study area

The study was carried out in the experimental area of the Bahia Foundation (Research and Development Support Foundation of Western Bahia), near the city of Luís Eduardo Magalhães, BA, Brazil (12° 5′35.16″ S and 45°42′40.30″ W), at an altitude of 960 m (Fig. 1). The soil is classified as a Typic Quartzipsament or Neossolo Quartzarenico according to the Brazilian Soil Classification System [21], with clay contents below 150 g kg^{-1} up to a depth of 80 cm (Table S1).

According to the Köppen classification, the regional climate is Aw, with a rainy season in summer and a dry season in winter [22]. The area, located in the Cerrado biome, has an average annual precipitation of 1511 mm and an average annual temperature of 24.2 °C, with the average temperature of the hottest month being 25.8 °C and the in coldest month 23.1 °C [23]. Monthly total precipitation and monthly mean air temperature (lines) over a 2-year period (2016/2017 and 2017/2018) are shown in Fig. 2.

The study was carried out in a field experiment started in 2012, whose objective is to evaluate the influence of different crop rotations and successions, under no tillage (NT) and conventional tillage (CT), in the production components of cotton (*Gossypium hirsutum*), soybean (*Glycine max* (L). Merr) and maize (*Zea mays* L.), in a total of 10 treatments (Table 1).

The experimental design was a randomized block with four replications, with the experimental plots measuring 20 m × 20 m, with row spacings of 50 cm for corn and soybeans and 76 cm for cotton. Annual fertilizations (at seeding and top dressing) in the experimental area are shown in Table S2.

The preparation of the area started in 2008 with a second clearing of the native vegetation of Cerrado. The site remained under fallow for 1 year, and in 2010–2011 growing season, the area was cultivated with cowpea (*Vigna unguiculata* (L.) Walp.). The area remained under fallow for another year. In 2012, soil sampling for chemical fertility was performed (Table S3). During the preparation of the area in 2012, subsoiling was carried out at a depth of 60 cm and 700 kg ha^{-1} of gypsum and 400 kg ha^{-1} of simple super phosphate were applied, followed by a disk harrow at 20-cm depth and harrowing at 10-cm depth. The field experiment was initiated in the 2012/2013 growing season, with the crop sequences shown in Table 1. In the CT plots, soil tillage consists of two operations that alternate: in 1-year subsoiling to a depth of 60 cm and two harrows to a depth of 0–10 cm, and, in the following year, a disk plowing to a depth 25 cm and two harrows of 0–10 cm.

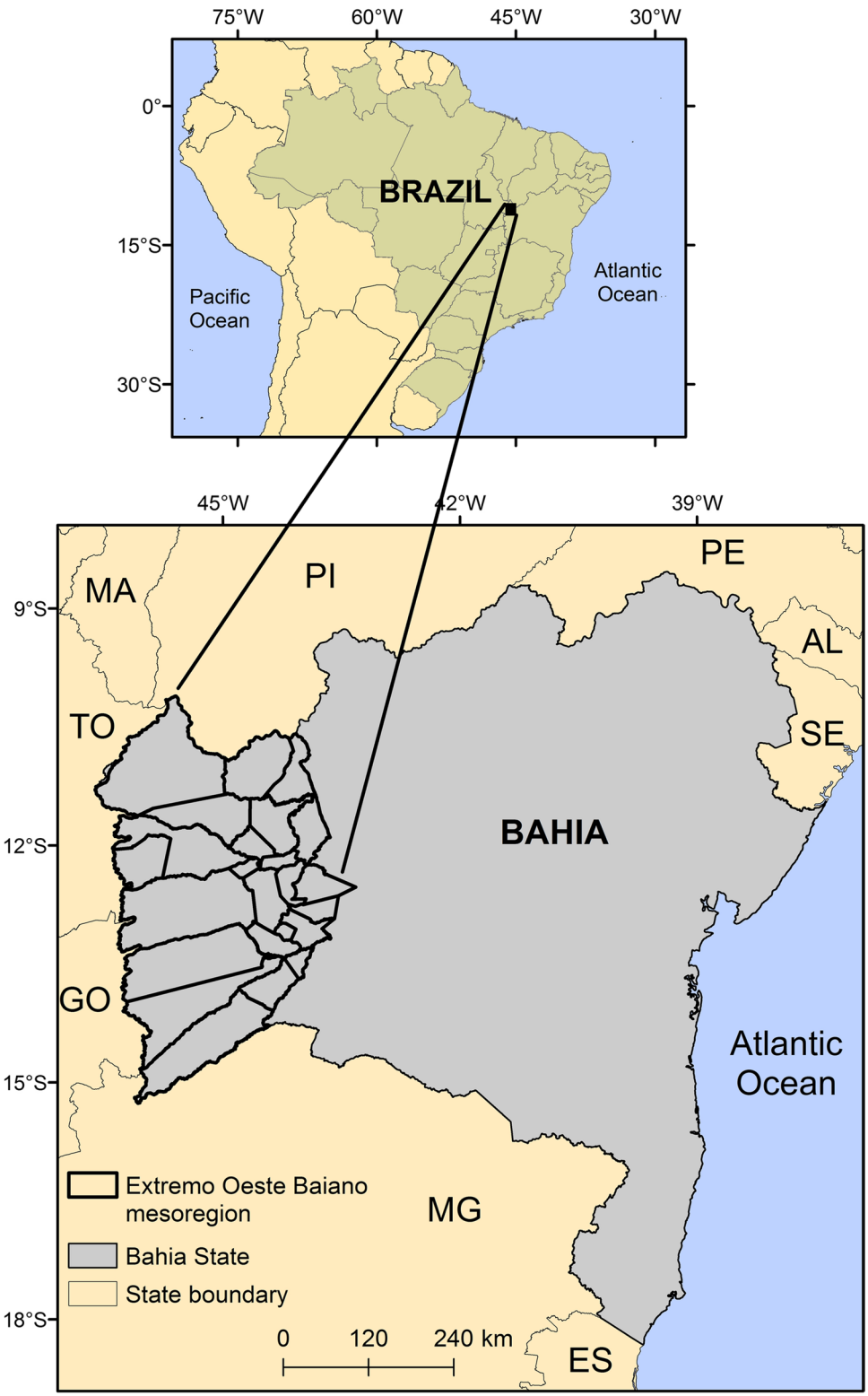

Fig. 1 Geographic location of the Western region of Bahia state, Brazil

Soil sampling and preparation

Soil samples were collected in March 2017 and February 2018, coinciding with the following phenological stages of the crops: soybean harvest, maize maturation, and cotton flowering. Soil samples were collected at three depths: 0–10 cm, 10–20 cm, and 20–40 cm, in the four replications of the ten selected treatments (total of 120 samples), at two points perpendicularly positioned to the 11th planting line. At each point, for each depth, five soil cores were

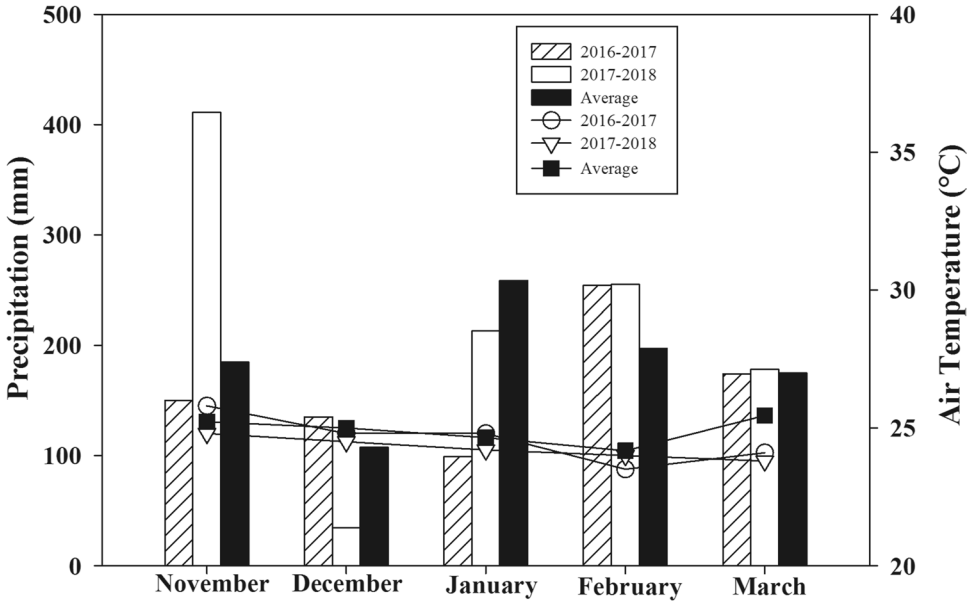

Fig. 2 Monthly total precipitation (bars) and monthly mean air temperature (lines) over a 2-year period (2016/2017 and 2017/2018). The averages of precipitation and mean temperature are relative of last 4 years before the soil sampling (2012–2013, 2013–2014, 2014–2015, and 2015–2016)

Table 1 Description of the cropping systems representing the ten experimental treatments

[a]Treatment	Year 1 2012/2013	Year 2 2013/2014	Year 3 2014/2015	Year 4 2015/2016	Year 5 2016/2017	Year 6 2017/2018
1	Soy	Soy	Soy	Soy	Soy	Soy
2	M	M	M	M	M	M
3	Ct	Ct	Ct	Ct	Ct	Ct
4	PM/Ct	PM/Ct	PM/Ct	PM/Ct	PM/Ct	PM/Ct
5	Soy	M + B	Soy	M + B	Soy	M + B
6	Ct	Soy/PM	M + B	Ct	Soy/PM	M/B
7	Soy/Crot	M + B	Ct	Soy/Crot	M + Brach	Ct
8	M + B	Ct	Soy/Crot	M + B	Ct	Soy/Crot
9	Ct	Soy/Crot	M + Crot	Ct	Soy/Crot	M + Crot
10	Soy/Sg	Ct	Soy/Sg	Ct	Soy/Sg	Ct

[a]Soil management: treatments 1 to 5 = conventional tillage and treatments 6 to 10 = no-tillage

Ct cotton, *B Brachiaria* (*Urochloa ruziziensis*), *Crot Crotalária ochroleuca* in succession to soybean and *Crotalaria spectabilis* in consortium with maize, *M* maize (*Zea mays* L.), *Sg* sorghum (*Sorghum bicolor* L.), *PM* pearl millet (*Pennisetum glaucum* L.)

collected equidistantly using a soil probe (5-cm diameter). The soil cores were collected perpendicularly to the planting row, with one core positioned in the middle of the planting row and the other two positioned on each side of the inter-row spacing. The soil samples were homogenized in plastic bags, transported to the Embrapa Cerrados Soil Microbiology Laboratory, and sieved in a 4-mm mesh. The samples for microbiological analysis were stored at 7 °C at field-moisture levels until the analyses were performed, about 1 week after collection. Plant debris and roots were carefully removed before the microbiological analyses. The soil samples for SOC and chemical properties were air-dried at room temperature for 72 h and sieved through a 2-mm sieve.

To assess the feasibility of using the FERTBIO concept [10] in sandy soils, in the sampling carried out in 2018, at the post-harvest stage, a portion of the air-dried soil samples for chemical analysis was also used to determine enzyme activities.

Soil analysis

The SOC content was measured using the Walkley–Black method [24] and calculated according to Jackson [25]. Chemical analyses (pH, Ca, Mg, K, and P) to characterize the areas were performed according to Embrapa [26]. The routine chemical analysis of the ten treatments in the 2 years of sampling is shown in Table S4.

The activities of β-glucosidase (E.C. 3.2.1.21), acid phosphatase (E.C. 3.1.3.2), and arylsulfatase (E.C. 3.1.6.1) were determined according to Tabatabai [12]. The method is based on the colorimetric determination of the p-nitrophenol released by the enzymes when 1 g of soil is incubated with 1 ml of a buffered solution containing synthetic substrate of p-nitrophenyl. During the 1-h incubation, toluene was not added. These three enzymes were selected according to their functions and importance in the C cycle (β-glucosidase), P cycle (acid phosphatase), and S cycle (arylsulfatase). Activity determinations for the three enzymes were carried out with field-moist soil samples (from 2017 and 2018) and also with air-dried soil samples (2018).

Statistical analyses

In order to evaluate the effect of the ten treatments on the EAs, in the three depths studied, an analysis of variance was used considering the mixed model by PROC MIXED of the SAS 9.1 program (SAS Institute, Cary, NC). When the differences were significant, the Student hypothesis test (t) ($P<0.05$) was used according to the model: $Yijk=\mu+ti+dk+t_id_k+bj+eij+\mathcal{E}ijk$, where μ = general data mean; ti = treatment i effect; dk = depth k effect; t_id_k = treatment × depth interaction; bj = block j effect; eij = experimental error; and $\mathcal{E}ijk$ = error generated by the depths. The model assumed that bj, eij, and $\mathcal{E}ijk$ were random effects.

For each treatment, a one-way analysis of variance was performed with sampling year (2017 and 2018) as the main effect, using the PROC GLM of the SAS software package (SAS Institute, Cary, NC). Sampling year means were compared using the least significant difference (LSD) test at 5% significance.

With the soil samples collected in February 2018 (soybean post-harvest stage) in order to assess the effects of air-drying in arylsulfatase, β-glucosidase, and acid phosphatase activities, a regression equation was calculated (R-software) to express the relationships between the field-moist and air-dried soil samples. A permutation test was used to determine the significance of slopes and R^2 determination coefficients. To determine if air-drying would influence the relation between EAs and SOC, correlation analyses between SOC and field-moist and air-dried soil samples also were performed (PROC CORR procedure of the statistical package SAS, SAS Institute, Cary, NC).

Orthogonal contrast analyses were performed to investigate if there were significant differences between grain yield, SOC, and EAs in the treatments under continuous cotton, soybean, and maize in CT, compared to the treatments under succession or rotation in NT, using the PROC GLM of the SAS software package (SAS Institute, Cary, NC).

Results and discussion

Interannual variation of SOC and soil enzymes

In the 2 years of monitoring (samplings conducted in February 2017 and March 2018), the monthly precipitation patterns and mean air temperature for the experiment site were consistent with the historical average of WB (Fig. 2).

Comparison of data obtained in 2017 and 2018 (both performed with field-moist soil samples) revealed that, with few exceptions, there was low interannual variability for SOC, arylsulfatase, β-glucosidase, and acid phosphatase activities, considering all treatments and soil depths evaluated (0–10, 10–20, and 20–40 cm) (Fig. 3). In treatments with rotations, the low interannual variability was observed even when soil sampling was performed under different crops in 2017 and 2018. Low variability of SOC results from the fact that it is a protected and recalcitrant material, whose changes in its contents demand time, being closely associated with the quantity and quality of organic material returned to the soil [27–30]. Although closely associated with the biological component of the soil, the temporal variations in the EAs over this 2-year period were minimal, reflecting the absence of significant fluctuations in climate variables (precipitation, temperature, and soil moisture) and in plant biomass production.

Low interannual variabilities of arylsulfatase and acid phosphatase were reported by Lopes et al. [19], over a 5-year period, in long-term field experiments in clayey Oxisols, regardless of the management system. Consistent and significant increases in β-glucosidase activity over time were observed only in treatments under NT and were attributed to its abiontic accumulation. Among the factors associated with the low temporal variation observed in soil microbial parameters the authors listed, the reduced differences in grain yield and plant biomass production over the years, the origin of soil samples (long-term field experiments), annual precipitation, and temperature patterns relatively consistent with the historic average and the length of establishment, i.e., the age of the cropping system [31, 32] which resulted in more accentuated treatment differences and reduced temporal variation over the years.

The FERTBIO soil sample concept for EA analysis in sandy soils

The feasibility of the FERTBIO soil sample concept for determinations of EAs in sandy soils was assessed with the samples collected in February 2018, when the treatments with the presence of soybean were at the harvesting stage. At the three soil depths evaluated (0–10 cm, 10–20 cm,

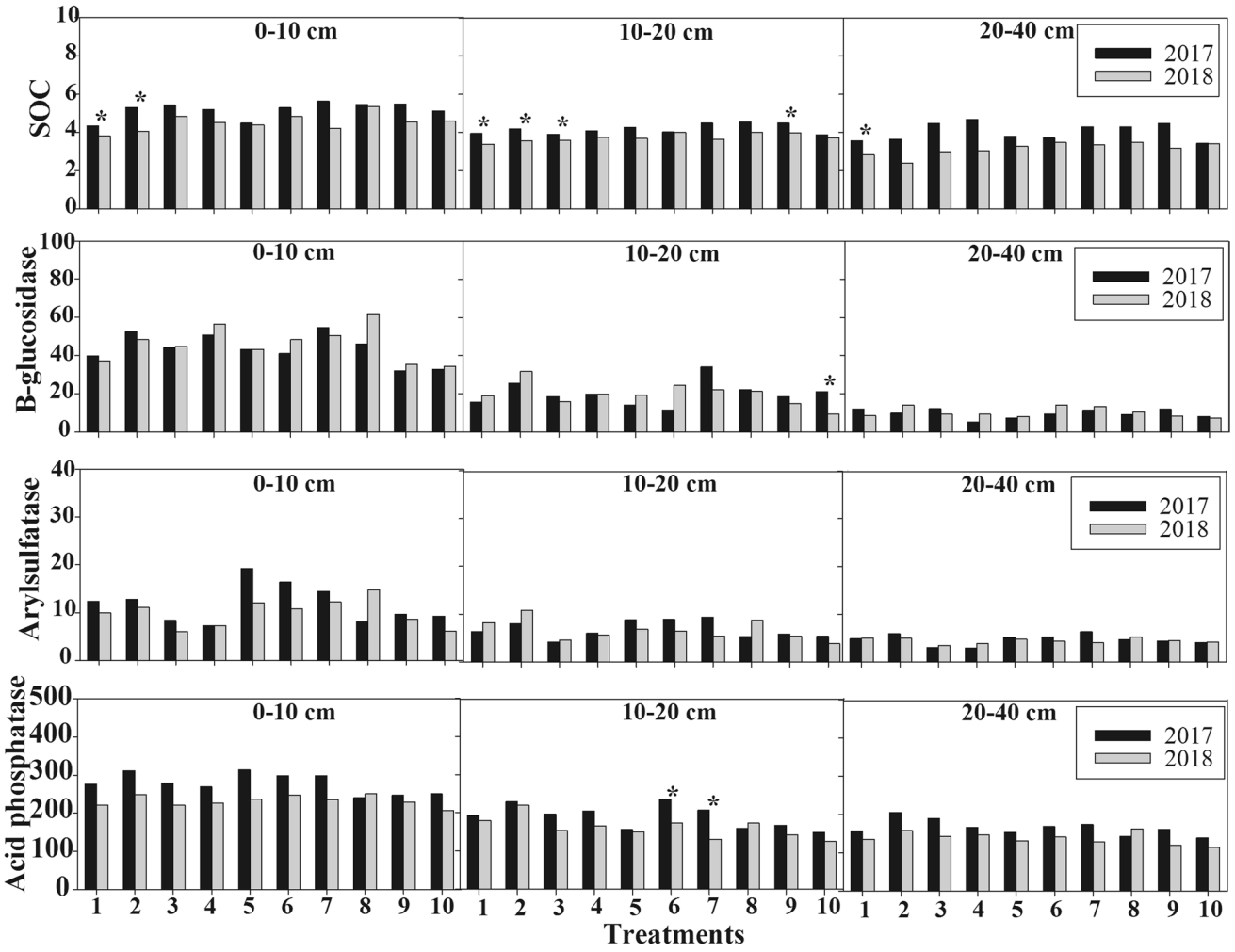

Fig. 3 Soil organic carbon (SOC) (g^{-1} kg^{-1}), β-glucosidase, arylsulfatase, and acid phosphatase activities (μg p-nitrophenol g^{-1} soil h^{-1}) in the treatments described in Table 1. In each treatment, * indicates differences (LSD, $P < 0.05$) between the 2017 and 2018 samplings. NS, non-significant (LSD, $P < 0.05$) at the 2017 and 2018 samplings

20–40 cm), linear regressions between the determinations performed with field-moist and air-dried soil samples confirmed that air-drying caused deviations from the initial levels of enzymatic activity (Fig. 4). Considering the deviation of a 1:1 ratio and the slope coefficients of 0.68, 0.62, and 0.77 for β-glucosidase, arylsulfatase, and acid phosphatase, respectively, average reductions promoted by air-drying soil samples at room temperature were 31%, 39%, and 25% for β-glucosidase, arylsulfatase, and acid phosphatase, respectively. Reductions in enzyme activity levels as a function of soil air-drying have been reported in the literature and are associated with cell disruption and enzymatic denaturation [10, 33–39]. Despite the observed EA reductions, the relationships between the two sets of samples (field-moist and air-dried) were positive and significant with R^2 of 0.94, 0.91, and 0.93 for β-glucosidase, arylsulfatase, and acid phosphatase, respectively (Fig. 4).

Overall, air-drying sandy soil samples did not interfere with the ranking of the treatments in relation to the activity levels of arylsulfatase, β-glucosidase, and acid phosphatase (Table 2). Due to the low clay and organic matter content of sandy soils, there was a fear that the severity of air-drying upon soil enzymes could reduce or even eliminate differences between treatments. By protecting soil enzymes against proteases action, adsorption of enzymes into clay particles and organic matter is one of the pathways of "soil memory" formation, as it reflects past generations of organisms that were present in that environment. The absence of significant changes in the ranking of the treatments in relation to the EAs observed showed that air-drying did not interfere with the ability of these biochemical indicators to access the "soil memory," enabling the use of the FERTBIO sample concept in this sandy soil.

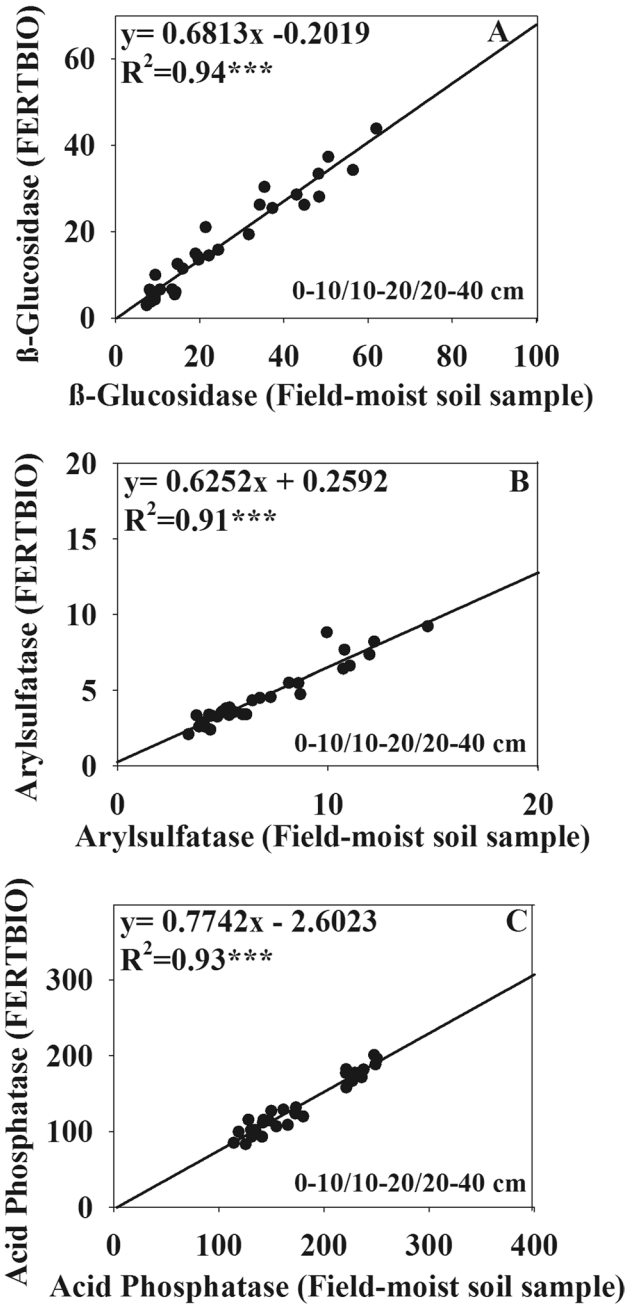

Fig. 4 Relationships between field-moist (*x*-axis) and air-dried soil samples (*y*-axis), for β-glucosidase (**A**), arylsulfatase (**B**), and acid phosphatase (**C**) activities (μg *p*-nitrofenol g^{-1} solo h^{-1}). Soil samples were collected in February 2018, in cotton production systems, in sandy soils of Western Bahia. The data points represent the average of the treatments, at three soil depths (0–10, 10–20, and 20–40 cm). All regressions parameters were significant ($P < 0.01$)

Air-drying did not interfere in the relations between SOC and EAs as evidenced by the correlation analyses between SOC and the two sets of EAs (air-dried and field moist) as shown in Table S5.

Short-term effects of different cropping systems on soil enzymes, SOC, and grain yield

Considering the low interannual variability in the 2017 and 2018 samplings, and the feasibility of the FERTBIO sample concept for this tropical sandy soil, the effects of different cropping systems under CT and NT on EAs and SOC were evaluated using only the 2018 FERTBIO sampling (air-dried soil samples collected at soybean harvesting).

The SOC and EA levels decreased as a function of sampling depth ($P < 0.001$), as shown in Table 2. Although these reductions were more accentuated for the NT treatments, they also were observed under CT. Overall, for β-glucosidase and SOC, the reductions followed the sch eme 0–10 cm > 10–20 cm > 20–40 cm. Arylsulfatase and acid phosphatase activities decreased rapidly below 10 cm. Reductions with soil depth were more pronounced for EAs, particularly arylsulfatase in treatments under NT, whose activity values at the 20–40 cm were close to zero (Fig. 3). Decreases in enzyme activities with increasing soil depth have been reported in the literature and are associated with root biomass and SOM reductions [40–43].

The magnitude of the impacts of 6 years of soil health improvements in this low organic matter sandy soil, varied according to the cropping systems evaluated. For a better understanding of these effects, in the 2018 sampling, a contrast analysis was performed with treatments under the same primary cash crop (Table 3). The contrast analyses compared intensively tilled continuous soybean, maize, and cotton, with regenerative cropping systems (with crop rotations/successions under no-till).

At the 0–10-cm depth, the comparison of continuous soybean (Soy-Soy) with M + B/Ct/Soy-Crot showed increases in β-glucosidase activity (76%), a carbon-cycling enzyme involved in plant residue degradation, and SOC (35%) (Table 3). In the same way, the comparison of continuous cotton (Ct-Ct) with the other three cropping systems with cotton as cash crop in the 2018 sampling (PM-Ct; Soy-Crot/M + B/Ct and Soy-Sorg/Ct) showed increases in β-glucosidase (38%) and arylsulfatase (200%) activities (Table 4). On the other hand, the contrast analysis between continuous maize (M-M) with the other three cropping systems with maize as cash crop (Soy/M + B; Ct/Soy-PM/M + B and Ct/Soy-Crot/M + Crot) showed significant effects only for SOC (19%), evidencing that, at this stage, maize monocrops systems, due to their higher biomass return, are less aggressive as compared to soy and cotton monocrops systems (Table 3). It is also noteworthy to point out that, in any of the contrasts at 0–10 cm, acid phosphatase was affected evidencing its lower capacity to detect early changes in soil functioning in response to different management practices. With one exception, no significant differences were detected by the contrast analyses at the 10–20-cm soil depth,

Table 2 Enzyme activities and soil organic carbon (SOC) determined in the soil sampling performed in February 2018, at the soybean harvesting stage, with field-moist soil samples (FM) and air-dried soil samples (AD)

Treatment	β-Glucosidase		Arylsulfatase		Acid phosphatase		SOC
	μg *p*-nitrofenol g^{-1} soil h^{-1}						g kg^{-1}
0–10 cm	FM	AD	FM	AD	FM	AD	
1	37cA	26dA	10bA	9aA	221abA	182abA	3.81dA
2	49bA	28cdA	11bA	6bA	249aA	189abA	4.05cdA
3	45bcA	26dA	6cA	3cA	221abA	158bA	4.68bA
4	57abA	34bcA	7bcA	4bcA	227abA	178abA	4.53bcA
5	43bcA	29cdA	12bA	7aA	238abA	182abA	4.71bA
6	48bA	33bcA	12bA	8aA	248aA	201aA	5.17aA
7	51bA	37abA	12bA	8aA	236abA	172abA	4.63bcA
8	62aA	44aA	15aA	9aA	251aA	196aA	5.14aA
9	35cA	30cdA	9bA	5bcA	230abA	178abA	4.54bcA
10	34cA	26dA	6cA	3cA	208bA	114cA	4.61bcA
10–20 cm							
1	19bB	15abB	8bcA	5aB	180bB	120abB	2.9bB
2	32aB	19aB	11aA	6aA	221aA	177aA	3.58aA
3	16bcB	11bB	5cA	3bA	155bcB	107abB	3.59aB
4	20bB	13bB	6cA	3bA	166bcB	109abB	3.75aB
5	20bB	14abB	7bcB	4abB	150bcB	127aA	3.70aB
6	25abB	16abB	6cB	4abB	173bB	124abB	3.99aB
7	23bB	14abB	6cB	4abB	131cdB	102abB	3.65aB
8	21bB	21aB	9abB	4abB	173bB	132aB	3.99aB
9	15bcB	13bB	6cB	3bB	143bcdB	115abB	3.97aB
10	10cB	10bB	4cA	3bA	126 dB	84bA	3.71aB
20–40 cm							
1	9aC	5aC	5aB	4aB	135abC	102abB	2.83bB
2	14aC	6aC	5aB	4aB	158aB	104abB	2.83bB
3	10aB	5aB	3aB	2aA	142abB	112abB	2.98abC
4	9aC	4aC	4aB	3aA	147abB	115abB	3.05abC
5	8aC	7aC	5aB	3aB	132abB	93abB	3.28abB
6	14aC	6aC	4aB	4aB	141abB	93abB	3.30abC
7	14aC	7aC	4aB	3aB	128abB	116abB	3.35abB
8	11aC	7aC	5aC	4aB	162aB	129aB	3.49aB
9	8aB	4aC	5aB	2aB	119bB	100abB	3.17abC
10	8aB	3aC	4aA	3aA	114bB	85bA	3.41aC

Values followed by lowercase letters in the same column indicate differences between treatments, at each depth ($P < 0.05$ by the Tukey test). For each treatment, values followed by capital letters indicate differences across the three soil depths ($P < 0.05$ by the Tukey test). The significant F values of the analysis of variance are shown in Table S6

emphasizing the adequacy of the 0–10-cm depth as a diagnostic layer for soil quality evaluations [17, 44]. Overall, these results are in agreement with studies in other regions indicating that more diverse cropping systems under no tillage can enhance soil microbial biomass and activity by increasing the residue input into the soil and reducing soil disturbance and erosion [45–49].

Compared to the treatments with monoculture under CT, in 2017/2018, a significant yield increase of 1358 kg/ha was observed for soybean under M + B/Ct/S-Crot rotation (Table 3). Although average yield increases of cotton lint (350 kg/ha) and maize (434 kg/ha) were observed in the rotation cropping systems, as compared to the monoculture treatments, the differences were not statistically significant (Table 3). The lack of more pronounced effects on grain yield reflects the difficulties in terms of the production of cover crops of biomass production. With the exception of *Brachiaria* grasses, dry matter production was below 5 t ha^{-1} (Table 4). This value is lower than 10 t ha^{-1} which is the minimum amount of biomass C-input recommended by Sá et al. [50] to maintain the steady state of C in the soil.

Table 3 Contrast analyses comparing intensively tilled continuous soybean, maize, and cotton with regenerative cropping systems (e.g., crop rotations/successions under no-till). All contrasts involve treatments under the same primary cash crop. Data from the soil sampling performed in February 2018, with air-dried soil samples (AD)

	B-Glu	Aryl	Acid phos	SOC	Yield
	—μg *p*-nitrofenol g^{-1} soil h^{-1}—			g kg^{-1}	kg ha^{-1}
Contrast					
Treat 1 × 8					
0–10 cm	25–44***	9–9 ^{ns}	182–196 ^{ns}	3.81–5.14***	3673–5031***
10–20 cm	15–21 ^{ns}	5–5 ^{ns}	120–132 ^{ns}	2.96–3.99***	-
Treat 2 × (5–6–9)					
0–10 cm	28–31 ^{ns}	7–7 ^{ns}	189–187 ^{ns}	4.05–4.81***	9191–9625 ^{ns}
10–20 cm	19–14 ^{ns}	6–4 ^{ns}	177–122***	3.58–3.89 ^{ns}	
Treat 3 × (4–7–10)					
0–10 cm	26–36***	3–6**	158–169 ^{ns}	4.69–4.58 ^{ns}	5247–5597 ^{ns}
10–20 cm	11–13 ^{ns}	3–4 ^{ns}	107–98 ^{ns}	3.60–3.70 ^{ns}	

For each contrast, values represent the average of the treatments being compared. Values followed by *** and ** differ from the intensively tilled continuous monocrop by the *F* test from the orthogonal contrast analyses at $P < 0.01$ and $P < 0.05$ probability levels

Treat treatment

Table 4 Grain yield and cover crop biomass obtained in the 2016/2017 and 2017/2018 seasons

Treat	Cropping systems		Yield		Cover crop shoot biomass	
	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
			— kg ha^{-1} —		**— kg ha^{-1} —**	
1	Continuous Soy	Soy	3418	3673	-	-
2	Continuous M	M	4166	9191	-	-
3	Continuous Ct	Ct	3869	5247	-	-
4	PM + Ct	PM + Ct	3888	5293	3571	2620
5	Soy	M + B	4416	9103	-	9941
6	Soy-PM	M + B	4714	9154	4433	9833
7	M + B/Ct	Ct	4098	5901	9866	
8	Ct	Soy-Crot	4387	5031	-	3664
9	Soy-Crot	+ Crot	4414	10,615	2466	4753
10	Soy-Sg	Ct	4232	5845	4700	-

Ct cotton, *B Brachiaria* (*Urochloa ruziziensis*), *Crot Crotalária ochroleuca* in succession to soybean and *Crotalaria spectabilis* in consortium with maize, *M* maize, *Sg* sorghum, *PM* pearl millet

Soils that have been managed to promote soil quality (e.g., minimum tillage, organic amendments, crop rotations/successions, etc.) would be expected to have higher microbiological activity [51]. This would be reflected in greater enzyme production. In the specific case of crop rotations, changes observed in soil properties are primarily related to the quantity and quality of plant residues and nutrients entering the soil, but they will also depend on the soil type [48, 52]. In addition to the length of establishment, i.e., period of the cropping system [31, 32] and difficulties in terms of plant biomass production, the lack of a more significant effect of the different cropping systems in sandy soils is associated with their low clay and organic matter contents. These characteristics make difficult to stabilize newly released enzymes and, therefore in time, build up the "soil memory" [35, 48]. In fact, Vinhal-Freitas et al. [52] reported a higher impact of land uses on enzyme activities in clayey soils, as compared to sandy soils, evidencing that soil textural class plays a major role in assessing differences between land use systems in the Brazilian Cerrado biome.

Low biomass cotton production systems have high potential profitability but, historically, have been detrimental regarding sustainability of natural resources. Previous studies in semiarid regions have emphasized the challenge of enhancing soil microbial communities in dryland cropping systems with low levels of biomass production due to low rain and extreme ambient temperatures [53, 54]. For example, studies by Acosta-Martinez et al. [48, 49] under different dryland cotton cropping systems, in an alkaline sandy soil in west Texas (USA), showed that the quality or quantity of residues returned to soil under different cotton cropping systems did not impact the properties of the sandy

soils after the first 3 years [48]. Depending on the cropping systems evaluated, it took up to 5 years to detect changes in total carbon, microbial biomass, and enzyme activities [49].

In the sandy soils of Western Bahia, the significant improvements in EAs observed due to the presence of cover crops and no-till can be an indication of changes in SOM, nutrient cycling, and C sequestration. These findings are ecologically significant because the enhancement of soil microbial communities, in these environments, is very challenging, as cover crops biomass production usually is low, due to limited precipitation, and because year-to-year climatic variability might prevent plant growth every growing season [49, 53, 54]. As pointed out by Acosta-Martinez et al. [49], increased enzyme activities in more diverse cropping systems, compared to monoculture, also reflect the importance of a management history of rotations in sandy soils to build a resilient extracellular enzymatic pool to sustain the biogeochemical potential under extreme adverse climatic conditions.

Conclusions

Regardless of the cropping systems and depths evaluated, SOC and EAs showed low temporal variation during the 2 years of monitoring. Air-drying sandy soil samples did not interfere with the ranking of the treatments regarding EAs and did not change the relations between SOC and EAs, thus proving the feasibility of using the FERTBIO soil sample concept in the sandy soils of Western Bahia. Five years after the beginning of the experiment, the presence of cover crops and no-till in cotton production systems promoted improvements in enzyme activities evidencing the importance of regenerative management practices, for the sustainability of agroecosystems in sandy soils.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-021-00606-z.

Acknowledgements We thank Clodoaldo A. de Sousa, Lucas F.L.S. Rolim, and Osmar T. Oliveira.

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Funding This work was financed by the Brazilian Agricultural Research Corporation (Embrapa, project 02.14.01.026.00) and partially financed by the National Council for Scientific and Technological Development (CNPq), Edital Universal (grant number 404764/2016-9), by the Research Support Foundation of the Federal District (FAPDF), Edital Demanda Espontânea 2016 (grant number 1355/2016) and by the MCTI/CNPq/CAPES/FAPS (INCT-MPCPAgro). I.C. Mendes and C.C. Figueiredo acknowledge a research fellowship from the CNPq.

Declarations

Conflict of interest The authors declare no competing interests.

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