Published February 25, 2013

Interpretation of Microbial Soil Indicators as a Function of Crop Yield and Organic Carbon

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An interpretative framework for microbial biomass C (MBC), basal respiration, and the activity of soil enzymes cellulase, β-glucosidase, arylsulfatase, and acid phosphatase was developed for the clayey Oxisols of the Brazilian Cerrado. Soil samples (0–10-cm depth) were collected from 24 treatments from three long-term experiments and analyzed to determine their microbial attributes and soil organic C (SOC). These treatments presented a large range of Mehlich-extractable P and cumulative corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] yields. The critical levels for the microbial indicators were defined based on criteria similar to those used in soil nutrient calibration tests. The microbial indicators were interpreted as a function of the relative cumulative yields (RCYs) of corn and soybean and the SOC using linear regression models. Adequacy classes for each microbial indicator as a function of the RCY and SOC were established based on the following criteria: ≤40%: low; 41 to 80%: moderate; and >80%: adequate. The critical levels equivalent to 80% of the RCY for MBC, basal respiration, cellulase, β-glucosidase, acid phosphatase, and arylsulfatase were: 375 mg C kg^{-1}, 90 mg CO_2–C kg^{-1}, 105 mg glucose kg^{-1} d^{-1}, 115 mg *p*-nitrophenol kg^{-1} h^{-1}, 1160 mg *p*-nitrophenol kg^{-1} h^{-1}, and 90 mg *p*-nitrophenol kg^{-1} h^{-1}, respectively. Similar critical levels were obtained when SOC was used as the interpretation criterion. The interpretation tables provided in this study establish, for the first time, reference values for the soil microbial indicators based on crop yields and constitute a first approximation. Their applicability to other conditions must be evaluated.

Abbreviations: MBC, microbial biomass carbon; RCY, relative cumulative yield; SOC, soil organic carbon.

There is growing evidence that soil microbial attributes are potential early indicators of changes in soil quality because they are more sensitive than a soil's chemical and physical properties (Miller and Dick, 1995; Bandick and Dick, 1999; Kandeler et al., 1999; Bending et al., 2004; Geisseler and Horwath, 2009; Peixoto et al., 2010). One of the major challenges in soil quality assessments using microbial indicators, however, is the difficulty in interpreting their individual values (Dick, 1992; Trasar-Cepeda et al., 1997; Gil-Sotres et al., 2005). Unlike the chemical indicators of soil fertility, for which the reference levels (low, medium, adequate, and high) are relatively well defined for each element and soil type (usually taking characteristics such as texture, organic matter content, and the management system into account), it is difficult to simply measure and interpret a series of microbial indicators independent of a comparative control or treatment (Dick, 1992).

The use of reference criteria (comparative assessments) has been suggested because the ideal values for the bioindicators can vary with climate, soil type, mineralogy,

Soil Sci. Soc. Am. J. 77:461–472
doi:10.2136/sssaj2012.0191
Received 14 June 2012.
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management, and land use. Two different approaches to establishing reference criteria for soil quality assessments have been proposed: (i) the use of native, undisturbed soils under climax vegetation and with minimal anthropogenic impacts; and (ii) the use of reference soils capable of maintaining a high level of productivity and environmental performance (Doran and Parkin, 1994; Gil-Sotres et al., 2005). Another alternative is to use temporal variation (dynamic assessment) to monitor soil quality bioindicators. In this case, the values determined for the bioindicators can be monitored to assess trends with time (Kandeler et al., 1999). In fact, the comparative and dynamic assessments are complementary, allow different rating scales, and each one has advantages and disadvantages (Gil-Sotres et al., 2005).

In New Zealand, an interpretative framework was developed for broad-scale soil quality monitoring, with appropriate target ranges for each soil indicator (including mineralizable N as an indicator of organic resources) and specific to certain land use and soil order combinations. The researchers used a set of interpretative response curves based on production and environmental goals and derived from a variety of sources using statistical approaches, agronomic studies, and expert opinion (Lilburne et al., 2004; Sparling et al., 2004; Sparling and Schipper, 2004). In the United States, the Soil Management Assessment Framework provides site-specific interpretations for soil quality indicators, including MBC, potentially mineralizable N, and β-glucosidase, based on the development of nonlinear scoring curves and their relationships with soil functions, which can be of three types: (i) more is better (upper asymptotic sigmoid curve), (ii) less is better (lower asymptotic sigmoid curve), and (iii) having a midpoint optimum (Karlen and Stott, 1994; Andrews et al., 2004; Stott et al., 2010). Indicators must be scored to interpret how each measure relates to the soil function of interest and to allow the indicators to be integrated by eliminating unit differences.

In soil fertility studies, the nutrient reference levels are determined using soil test calibrations in which the nutrient content (supplied either by the soil or by fertilizers) is related to some plant indicator, such as production. The yield responses to various rates of applied nutrients are then related to the quantity of available nutrients in the soil as indicated by the soil test (Tisdale et al., 1993). These relationships are used to determine the critical level, which is defined as either the concentration of the nutrient in the soil or plant tissue above which little or no increase in production is expected (Tisdale et al., 1993) or the nutrient concentration that separates populations of low and high probabilities of response to the addition of the nutrient. In many cases, the critical level is also defined as the nutrient concentration that is necessary to reach either 80 or 90% of the maximum economic yield (Maia et al., 2001; Cantarutti et al., 2007). Curve response experiments conducted under field conditions are essential to establish the equations used to provide fertilizer recommendations that will optimize crop yield, maximize profitability, and minimize the environmental impact of nutrient use (Tisdale et al., 1993). The different levels of soil nutrients are adjusted for crop yields using mathematical (regression) models, such as the Mitscherlich, quadratic, and exponential models. The soil test values are then classified into sets of interpretative categories that describe the relative crop availability of a given nutrient (low, medium, adequate, and high). A soil with a high test value will require little or no addition of nutrients compared with one with a low test value.

When the influence of factors not directly related to soil quality (climatic adversities, plant genotypes, and the occurrence of pests and diseases) is minimized, crop yield can be considered a field indicator for evaluating sustainability that takes the satisfaction of the farmer into account (Gomez et al., 1996). Given the complexities of yield responses to critical soil parameters, crop yield can also be considered an integrator of soil indicators, such as organic matter, topsoil depth, infiltration, aggregation, pH, electrical conductivity, suspected pollutants, and soil respiration (Arshad and Martin, 2002). Therefore, it can be hypothesized that specific crop yield situations (ranging from low to high) could be useful in developing a guideline for the interpretation of individual soil quality indicators.

In this study, we tested this hypothesis and developed an interpretative framework for MBC, basal respiration, and the activity of soil enzymes β-glucosidase, cellulase, arylsulfatase, and acid phosphatase based on the use of the principles of soil nutrient calibration tests. The microbial indicators were interpreted as a function of the cumulative corn and soybean yields and SOC in a clayey Oxisol of the Brazilian Cerrado. A set of three long-term field experiments in which P fertilization management was used to modulate the crop yields and SOC provided the ideal conditions to model these relationships.

MATERIALS AND METHODS

Study Area

The study area was located at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Centro de Pesquisa Agropecuária dos Cerrados (Cerrados Research Center), located close to the city of Planaltina, DF, Brazil (15°35′30″ S and 47°42′0″ W, at an altitude of 1175 m). The soil is a very fine, mixed isothermic Rhodic Haplustox (a Typic Dystrophic Red Latosol, according to the Brazilian soil classification system). According to the Köppen classification, the regional climate is Cwa, which corresponds to a typical savanna climate with 1500 mm of mean annual precipitation and two well-defined seasons: dry, from May to September, and rainy, from October to April. The maximum and minimum yearly average temperatures are 26.4 and 15.9°C, respectively.

Twenty-four treatments from three long-term field experiments were selected based on the historical cumulative grain yields of soybean and corn. These experiments, which were designed to study the management of P fertilizers using various sources, application rates, and placement methods, resulted in cumulative grain yields ranging from 3385 to 96,553 kg ha^{-1} during a 17-yr period for Exp. I and a 12-yr period for Exp. II and III (Table 1). Granular triple superphosphate was the only P source used in the 24 treatments selected for the present study.

Table 1. Description of the 24 selected treatments from the long-term Exp. I, II, and III and their respective accumulated crop yields (ACY), relative cumulative yield (RCY), Mehlich-extractable P, and soil organic C (SOC) contents in the 2011 sampling.

Treatment	P_2O_5 application rate		Placement†	Management‡	ACY	RCY	P	SOC
	1st year	2nd year and after						
	kg ha^{-1} yr^{-1}				kg ha^{-1}	%	mg kg^{-1}	g kg^{-1}
				Experiment I				
1	0	0	–	CT/MIL	3.385	4	0.9	12.7
2	80	0	Br	CT/MIL	7.371	8	1.0	13.5
3	160	0	Br	CT/MIL	13.627	14	1.0	14.5
4	240	0	Br	CT/MIL	17.997	19	1.2	14.3
5	480	0	Br	CT/MIL	30.325	32	1.3	15.0
6	80	80	Br/Ba.	CT/MIL	88.609	93	9.7	19.1
7	240 + 80	80	Br + Ba/Ba	CT/MIL	92.662	97	10.9	19.6
8	80	80	Ba	NT/MIL	96.553	99	10.4	20.5
				Experiment II				
9	0	0	–	NT/LEG	51.484	47	2.8	15.1
10	50	50	Ba	NT/LEG	86.201	90	8.5	17.5
11	100	100	Ba	NT/LEG	91.486	99	14.9	16.5
12	0	0	–	NT/MIL	51.173	56	5.5	17.2
13	50	50	Ba	NT/MIL	87.334	93	11.5	19.4
14	100	100	Ba	NT/MIL	91.493	99	32.9	19.2
15	0	0	–	CT/MIL	44.153	54	3.6	17.3
16	50	50	Ba	CT/MIL	84.156	93	11.9	19.7
17	100	100	Ba	CT/MIL	92.858	97	33.1	19.0
				Experiment III				
18	0	0	–	NT/MIL	5.443	7	1.1	12.7
19	0	80	Ba	NT/MIL	75.508	93	8.8	18.7
20	240	0	Br	NT/MIL	28.936	36	1.4	14.7
21	240	80	Br/Ba	NT/MIL	79.328	98	17.5	19.2
22	0	40	Ba	NT/MIL	58.978	73	6.9	17.3
23	0	60	Ba	NT/MIL	66.451	82	8.2	19.1
24§	0	80	Ba	NT/MIL	40.594	50	2.7	16.6

† Br, broadcast; Ba, band applied; Br/Ba, broadcast in the first year and band applied thereafter; Br + Ba/Ba, 240 kg ha^{-1} of P_2O_5 broadcast along with 80 kg ha^{-1} of band-applied P_2O_5 in the first year and annual band applications of 80 kg ha^{-1} of P_2O_5 from the second year and after.

‡ CT/MIL, conventional tillage with pearl millet as cover crop; NT/MIL, no-till with pearl millet as cover crop; NT/LEG, no-till with mucuna as cover crop.

§ Annual fertilization with 80 kg ha^{-1} of P_2O_5 beginning in 2009–2010 and 2010–2011.

Table 2 presents the particle size distribution and chemical analyses of soil samples collected at a depth of 0 to 20 cm before establishing the three long-term field experiments.

Experiment I was established in 1994 to evaluate P fertilizer sources, placement methods (band or broadcast), and different application rates under no-till and disk-plow tillage systems. The experimental design was a randomized complete block with three replicates. The plots were 8 by 4 m, with row spacings of 50, 80, and 20 cm for soybean, corn, and pearl millet [*Pennisetum glaucum* (L.) R. Br.], respectively. The area was cleared in 1976 and left fallow until 1985, at which time it was cropped for 3 consecutive yr with green manure species; it was again left fallow from 1988 until 1994. The experiment was initiated in 1994–1995, at which time soybean was cultivated until 2003 without cover crops. Beginning in 2003, pearl millet was used as the winter cover crop and corn was cultivated in 2004–2005. The plots were subsequently in a corn–soybean rotation that was managed for maximum yields (Sousa and Lobato, 2004).

Experiment II was established in 1999 to evaluate P fertilizer sources and application rates under three management systems: no-till with pearl millet as the cover crop, no-till with mucuna [*Mucuna aterrima* (Piper & Tracy) Holland] as the cover crop,

Table 2. Soil chemical properties and particle size analysis in the study area. Samples were collected from the 0- to 20-cm depth before the establishment of the three experiments. For the native Cerrado areas, samples were collected in January 2011 from a depth of 0 to 10 cm.

Study area	pH (H_2O)	H + Al	Al^{3+}	Ca + Mg	K	P	Soil organic C	Clay	Sand	Silt
		mol$_c$ m^{-3}			mg kg^{-1}		g kg^{-1}			
Exp. I	5.4	52.7	0.8	44.2	1.1	1.0	16.2	640	270	90
Exp. II	5.4	36.6	0.0	45.4	1.5	10.5	13.3	571	357	72
Exp. III	4.5	81.6	14.0	4.0	1.0	1.2	16.2	540	410	50
Cerrado I	5.0	78.5	10.4	8.9	1.0	1.0	19.9	620	310	70
Cerrado II	5.0	75.3	13.8	4.1	0.8	1.0	17.9	580	350	70
Cerrado III	5.1	89.8	12.1	1.9	0.8	0.7	21.1	660	250	90

and conventional tillage with pearl millet as the cover crop. The experimental design was a randomized complete block with split plots and three replicates (the management systems were the main plots and P fertilizer treatments were the subplots). The subplots were 11 by 4.5 m, with row spacings of 45, 75, 20, and 60 cm for soybean, corn, pearl millet, and mucuna, respectively. The area was cleared in 1976 and was under pasture until 1996; this area was then cultivated with soybean (1996–1997 and 1997–1998) followed by corn (1998–1999). The experiment was initiated in 1999–2000, with a rotation of soybean–corn–winter cover crop that was managed for maximum yields (Sousa and Lobato, 2004).

Experiment III was established in 1999 to evaluate P fertilizer sources, application rates, and placement methods (band or broadcast). The experimental design was a randomized complete block that was organized as a complete factorial with three replicates. The plots were 11 by 4.5 m, with row spacings of 45, 75, and 20 cm for soybean, corn, and pearl millet, respectively. The area was cleared in 1976 and left fallow until 1999, at which point the experiment was initiated. Soybean was cultivated in the first 2 yr (1999–2000 and 2000–2001) and the rotation of soybean–corn was initiated in 2001–2002, with pearl millet as a winter cover crop; the plots were managed under no-till for maximum yields (Sousa and Lobato, 2004).

Three nearby areas with native Cerrado vegetation (with the same soil type and hillslope position) were also included in the study as reference for the original soil conditions. The particle size distribution and chemical analyses of the soil samples collected in these areas in January 2011 at a depth of 0 to 10 cm are also presented in Table 2.

Soil Sampling and Preparation

The soil samples were collected in January 2011 at the corn tasseling stage. In each plot of the 24 selected treatments, soil samples were collected at a depth of 0 to 10 cm at four different points to form a composite sample. At each point, seven soil cores were collected equidistantly using a soil probe (5-cm diameter); the soil cores were collected perpendicular to the planting row, with one core positioned in the middle of the planting row and the other three positioned on each side of the interrow spacing. These subsamples were homogenized in large plastic bags, transported to the laboratory, and sieved through a 4-mm sieve. A total of 72 samples were collected for the three experiments (24 treatments, each with three replicate plots). In the areas under the native Cerrado vegetation, three 10- by 10-m plots (replicates) were defined along a transect (east–west) and 10 soil samples were randomly collected to form a composite from each plot. Samples for the microbiological analyses were stored at 7°C at field moisture until the analyses were performed within a period of 1 wk after sampling. The plant debris and roots were carefully removed before the microbiological analyses. The soil samples for the SOC determination were air dried at room temperature for 72 h and sieved through a 2-mm sieve.

Soil Analyses

The SOC content was measured using the Walkley–Black method (Nelson and Sommers, 1996) and calculated according to Jackson (1958). The available P was extracted with Mehlich-1 extractant (0.0125 mol L^{-1} H_2SO_4 + 0.05 mol L^{-1} HCl), and the P concentration was determined by colorimetry (Embrapa, 1997).

The basal soil respiration (or readily mineralizable C) was determined by incubating the soil samples (20 g) in airtight canning jars (500 mL). Before the incubation, distilled water was added to the soil samples to reach 55% water-filled pore space, and scintillation vials containing 10 mL of 0.3 mol L^{-1} KOH were placed inside the canning jars. The samples were incubated in the dark for 7 d at 28°C. The amount of evolved CO_2–C was determined by titration with 0.1 mol L^{-1} HCl after precipitation with 1.0 mol L^{-1} $BaCl_2$. This analysis was performed using three analytical replicates.

The soil MBC was determined using the chloroform-fumigation–extraction method described by Vance et al. (1987). The same soil samples used for the basal respiration analysis were used as the unfumigated (UF) controls immediately after the 7-d incubation period. Another three replicates were also preincubated under the same conditions; on the fifth day, these samples were fumigated (F) at room temperature for 48 h in desiccators containing 20 mL of ethanol-free chloroform. The UF controls were kept at 28°C. After fumigation, the microbial C was extracted with 50 mL of 0.5 mol L^{-1} K_2SO_4 from all of the samples. The organic C was quantified using the potassium dichromate oxidation method (Jenkinson and Powlson, 1976) and subsequent back-titration of unreduced dichromate. The amount of MBC was determined by the difference between the C extracted from the F and UF controls using a k_{EC} of 0.35 (Joergensen, 1996).

The β-glucosidase (E.C. 3.2.1.21), acid phosphatase (E.C. 3.1.3.2), and arylsulfatase (E.C.3.1.6.1) activities were determined according to Tabatabai (1994). Due to their short incubation periods (1 h), toluene was omitted from the assays. The cellulase (E.C. 3.2.1.4) activity was determined according to Schinner and von Mersi (1990). These four soil enzymes were selected for their roles in the C cycle (β-glucosidase and cellulase), P cycle (acid phosphatase), and S cycle (arylsulfatase).

Data Analyses

A variable defined as the relative cumulative yield (RCY) was calculated to express the cumulative yield of corn and soybean as a percentage of the greatest cumulative yield obtained in each of the three experiments. This relative variable was used to express the area productivity using the historical data of the three experiments evaluated, which had different durations and different rotations.

Linear regression equations were defined to express the relationship between the RCY (dependent variable) and SOC and the individual soil microbial indicators (MBC, basal soil respiration, and β-glucosidase, cellulase, arylsulfatase, and acid phosphatase activities). When necessary, a quadratic component

was added to the models to attain a homogeneous variance and the best fit. Similarly, regression equations were calculated for the relationship between SOC and the microbial indicators. The microbial indicators were also correlated with the SOC. The relationships between the Mehlich-extractable P and RCY and between Mehlich P and SOC were graphed using the Mitscherlich model. All of the statistical analyses were performed using the SAS software package (SAS Institute).

Two approaches were used to produce the interpretation tables for the microbial indicator values. The first approach was based on the relationships between the RCY and each soil microbial indicator. Accordingly, values of the bioindicator that were higher than an RCY of 80% were considered adequate, assuming that an RCY of 80% corresponds to the production of maximum economic efficiency (similar to the critical level concept for soil nutrients). Values of the bioindicator corresponding to an RCY between 41 and 80% were classified as moderate, and values corresponding to an RCY of ≤40% were classified as low (inadequate). This approach was analogous to that used by Sousa and Lobato (2004) for the interpretation of the Mehlich P levels in Cerrado soils. In soil fertility studies, these classes have been associated with a decreased probability of an economic response to fertilization (Tisdale et al., 1993).

The second approach was based on the mathematical relationship between the RCY and SOC content. Similarly to the first approach, the interpretative classes for SOC content were defined (RCY ≤ 40%: low; RCY 41–80%: moderate; RCY > 80%: adequate). After the definition of the limits of the classes for SOC content, we used them in the regression equation models defined to express the relationship between SOC and the microbial indicators to obtain the interpretative classes for each microbial indicator. This approach was intended to express the relationship between the RCY and microbial indicators indirectly, assuming a high correlation between the RCY and SOC content.

RESULTS AND DISCUSSION

Soil Phosphorus and Relative Cumulative Yield

Cerrado soils under natural conditions have a low P availability in the soil solution due to their high capacity to retain this nutrient in the solid phase. This fact makes the use of P fertilizers necessary to raise the soil P levels for economic crop production (Sousa and Lobato, 2004). This dependence is clearly expressed in Fig. 1, in which a variation of the soil P levels from 0.9 to 36 mg kg^{-1} was associated with a variation from 3 to 100% in the RCY of corn and soybean. The mathematical model that best expressed this relationship was the Mitscherlich model ($R^2 = 0.94$, $P < 0.001$). Accordingly, as the soil Mehlich P levels increased, the RCY also increased in a curvilinear manner, reaching an equilibrium plateau (Fig. 1).

The equation displayed in Fig. 1 showed that the equivalent soil P level for 80% of the RCY was approximately 8 mg kg^{-1}, which is similar to the value reported by Sousa and Lobato (2004) for unirrigated cropping systems in clayey Oxisols in the Cerrado region at a 0- to 20-cm depth.

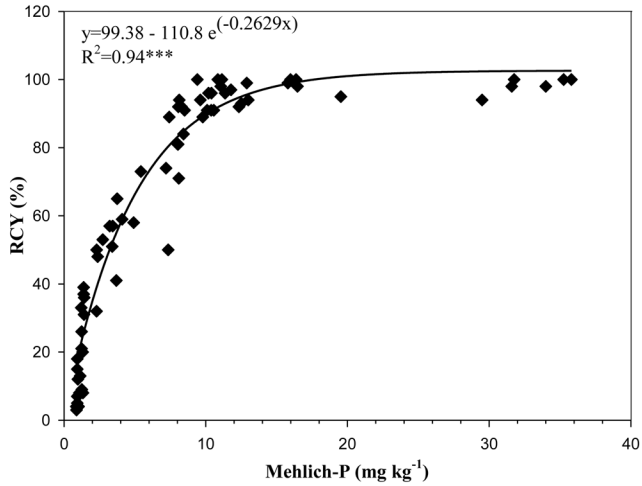

Fig. 1. Relationship between the relative cumulative yield (RCY) and soil Mehlich-extractable P. The data points represent the three field replicates of the 24 selected treatments. ***Significant at $P < 0.001$.

Soil Organic Carbon and Relative Cumulative Yield

The significant increases in the RCY in response to soil P levels resulted in a linear increase in the SOC content (Fig. 2). Just as the P application increased the crop yield, it also increased the return of organic matter to the soil from both the aboveground residue and root turnover, thus increasing the SOC content. Similar results were reported by Graham et al. (2002) in long-term sugarcane (*Saccharum officinarum* L.) fields in South Africa and by Masto et al. (2007) in a long-term nutrient and crop management experiment in India.

In addition to the C added by the cover crops, the total amount of C added to each plot was estimated assuming that the harvest index for grain crops (the ratio of harvested grain to the total aboveground biological yield) is approximately 50% (Unkovich et al., 2010), i.e., for each megagram of grain yield, 1 Mg of shoot dry matter is produced, and assuming that the shoot/root ratio in soils for cereal crops is 30% (Balesdent and Balabane, 1996; Bolinder et al., 1997; Kisselle et al., 2001). Based on these assumptions and the total grain production data (Table 1), it can be estimated that 4.4 to 125 Mg ha^{-1} of crop residues (leaves, stems, and roots) was added to the soil during the 12 or 17 yr since the beginning of the experiments. These residue input quantities resulted in SOC levels ranging from 11.7 to 21.1 g kg^{-1} (Fig. 2).

Soil Phosphorus, Soil Organic Carbon, and Microbial Indicators

The significant increases in the amount of crop residues added by the different cropping systems in response to P fertilizers led to an increase in SOC content. A variation of the soil P levels from 0.9 to 36 g m^{-3} was associated with variation from 11.7 to 21.1 g kg^{-1} of SOC (Fig. 3). The mathematical model that best expressed this relationship was the Mitscherlich

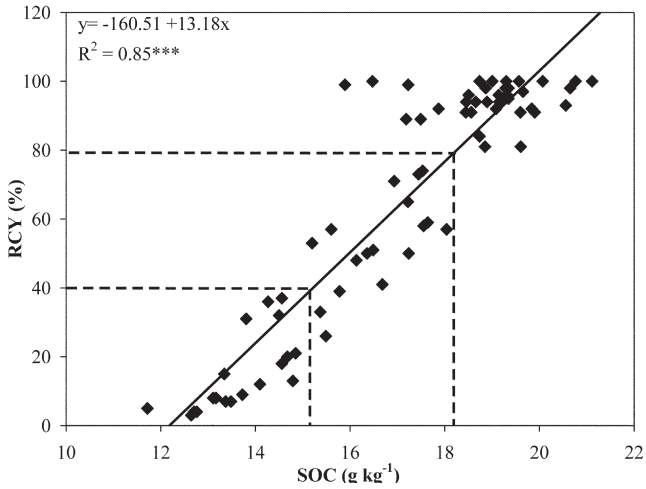

Fig. 2. Relationship between the relative cumulative yield (RCY) and soil organic C (SOC) content. The data points represent the three field replicates of the 24 selected treatments. Dashed lines represent the limits of the interpretative classes: ≤40% RCY: low; 41 to 80% RCY: moderate; and >80% RCY: adequate. ***Significant at $P < 0.001$.

model ($R^2 = 0.81$, $P < 0.001$). Accordingly, as the soil Mehlich-P levels increased, the SOC also increased in a curvilinear manner, reaching an equilibrium plateau (Fig. 3), demonstrating that P fertilization in Cerrado soils is an important practice not only to promote increased grain crop yields but also to maintain or even increase the SOC content.

Soil organic matter stocks are directly related to the amount of C added by the cropping system. The importance of N inputs in forest and agroecosystems to enhance C sequestration is also well established (Bayer et al., 2006; Hanson et al., 2007). Considering that P is a limiting factor for agricultural production in Cerrado Oxisols (Sousa and Lobato, 2004), however, a similar effect occurs because in systems with adequate application of P fertilizers, there is a greater production of plant biomass and therefore increased incorporation of C in the soil through photosynthesis and of N through biological N_2 fixation by legumes. Sousa et al. (2010) verified SOC contents at the 0- to 20-cm depth ranging from 14 to 22 g kg^{-1} due to differences

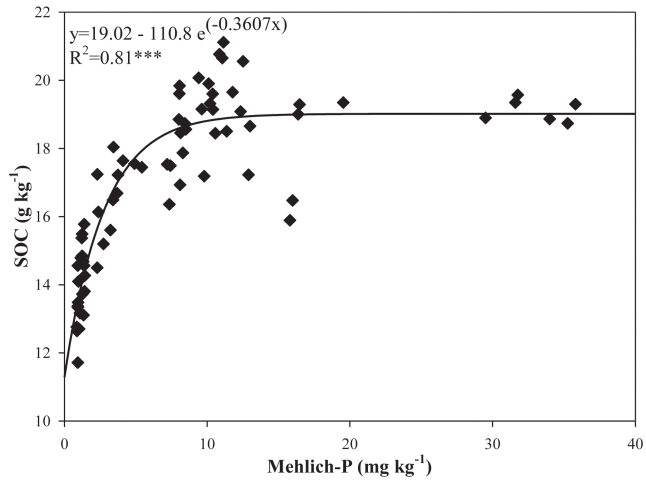

Fig. 3. Relationship between soil Mehlich-extractable P and soil organic C (SOC) content. The data points represent the three field replicates of the 24 selected treatments. ***Significant at $P < 0.001$.

Table 3. Pearson's correlation coefficients for the relationships between soil attributes and the relative cumulative yield (RCY), soil organic C (SOC) content, and Mehlich-extractable P in the 24 selected treatments. All coefficients were significant at $P < 0.001$.

Soil attribute	RCY	SOC	Mehlich P
SOC	0.92	–	–
Mehlich-P	0.72	0.63	–
Microbial biomass C	0.76	0.74	0.46
Basal respiration	0.87	0.83	0.57
Cellulase	0.77	0.80	0.47
β-Glucosidase	0.84	0.85	0.59
Acid phosphatase	0.74	0.79	0.55
Arylsulfatase	0.82	0.87	0.65

in the inputs of plant residues as a function of P fertilizer applications in annual cropping systems and pastures. Similar results were also reported by Nunes et al. (2011).

Pearson's correlation coefficients for the relationships between the microbial indicators and Mehlich-P ranged from 0.46 to 0.65 and were lower than those obtained for RCY and SOC (both ranging from 0.74–0.87; Table 3). These relationships suggest that in these three long-term field experiments, modulation of crop yields and residue production through different P-fertilization strategies resulted in a range of SOC levels in the soil, which in turn affected microbial communities. Although soil is a major reservoir of organic C (Bossio and Scow, 1995), the lack of available C is the most common limiting factor for microbial growth (Demoling et al., 2007).

The determination of which nutrient is directly limiting microbial growth in the soil is not trivial (Demoling et al., 2007); however, the higher levels of MBC in the native Cerrado soils compared with those found in soils under agroecosystems (Mendes et al., 2012; Peixoto et al., 2010) and the lower Pearson's correlation coefficients for the relationships between the microbial indicators and Mehlich P (Table 3) suggest that for this particular soil type, P additions do not limit microbial growth to the same extent as they limit the development of agricultural crops.

Relationships between Microbial Indicators with Relative Cumulative Yield and Soil Organic Carbon

The microbial indicators were significantly and positively correlated with both the RCY and SOC content (Table 3). Pearson's correlation coefficients ranged from 0.74 to 0.87 ($P < 0.001$), with the highest correlations obtained for basal respiration, β-glucosidase and arylsulfatase activities.

Because crop yields can be influenced by numerous factors that are not related to soil quality, such as climate, plant genotype, and the occurrence of pests and diseases, correlations between the microbial indicators and crop yield are not often found in the literature (Verstraete and Voets, 1977; Dick et al., 1988; Hungria et al., 2009). We attribute the high correlations obtained in the present study to the unique opportunity identified in these long-term field experiments where: (i) in the 24 selected treatments, the management of P fertilizers made it possible to modulate the

crop yields (Fig. 1) and the SOC content (Fig. 2), and (ii) the influence of factors not related to soil quality was minimized by the use of the long-term cumulative yield, expressed as the RCY, rather than a single yield measurement. Therefore, the RCY reflected all of the changes in the soil throughout the duration of these long-term field experiments more accurately than would have single yield measurements.

Soil organic matter is the most important component of soil fertility in the highly weathered Cerrado soils and is considered a key indicator of soil quality due to its positive influence on biological, chemical, and physical soil attributes (Karlen et al., 2001; Sparling et al., 2003). In the present study, the relationships observed between the six microbial indicators with the RCY and SOC content show that highly productive soils also present high levels of microbial biomass and activity, which in addition to the increased SOC contents result in high-quality soils. This finding underlies the interpretative tables for the microbial indicators described below.

Interpretation of Microbial Indicators as a Function of Relative Cumulative Yield and Soil Organic Carbon

The adjusted linear regression models for the RCY as a function of each of the soil's microbial indicators are presented in Fig. 4. Quadratic linear models were adjusted for all of the microbial indicators. The models with the best adjustment based on the R^2 values were for arylsulfatase activity (0.84) and soil basal respiration and β-glucosidase activity (both 0.79), whereas lower R^2 values were obtained for MBC and cellulase and phosphatase activities, although their model parameters were still significant ($P < 0.001$). These relationships demonstrate that the RCY increases with increasing soil microbial biomass and activity to an optimum range, above which further increases in these indicators are not associated with a higher RCY. Therefore, highly productive soils may present a large range of values for MBC, respiration, β-glucosidase, cellulase, phosphatase, and arylsulfatase, yet these soils may show a steep decrease in productivity below this optimum range.

In our data set, we observed a good correlation between accumulated crop yields and SOC. Given that SOC content is a component of many of the soil functions related to soil quality (retention and cycling of nutrients, physical stability and support, water retention, buffering and filtering of potentially toxic materials, and maintenance of biodiversity and habitat), the fitted models in Fig. 4 also demonstrate the high sensitivity of the bioindicators to changes in soil quality, evidenced here by changes in the RCY. For instance, the change in soil β-glucosidase activity from the lowest to highest RCYs was equivalent to 716%; this relative change was equivalent to 1311 and 3143% for soil basal respiration and arylsulfatase activity, respectively. Based on Fig. 2, the variation in the SOC content along the RCY gradient was only 55%, which is 57 times lower than that observed for arylsulfatase activity. These results corroborate other studies that showed the greater sensitivity of microbial soil attributes to reflect changes in management practices compared with SOC (Miller and Dick, 1995; Bandick and Dick, 1999; Kandeler et al., 1999; Bending et al., 2004; Geisseler and Horwath, 2009; Peixoto et al., 2010). These results also suggest that some microbial attributes are more sensitive than others, which can be used as a criterion for selecting bioindicators in soil quality evaluation programs.

Based on the fitted models shown in Fig. 4, it was possible to elaborate a first attempt to interpret the individual values of the microbial indicators as a function of the RCY (≤40% RCY: low; 41–80% RCY: moderate; and >80% RCY: adequate) by calculating the limits of the three interpretative classes. The classes for each microbial indicator are presented in Table 4 and displayed graphically in Fig. 4. Although the definition of the limits of the interpretative classes was arbitrary, it illustrates the concept of the probability of a response (Tisdale et al., 1993) and, for the first time, establishes reference values for the soil microbial indicators in the Red Latosols of the Cerrados region based on crop yields. This interpretative table may provide information on the effectiveness of selected farming systems and land use practices and their impacts on soil quality. For example, a low test value for the microbial indicators may be an indication of inadequate management practices. According to Arshad and Martin (2002), these critical limits (established here as 80% of the RCY) can also be understood as the desirable range of values for a selected soil indicator that must be maintained for normal functioning of the soil ecosystem health.

Figure 5 shows the individual relationships between the six microbial indicators and SOC contents obtained in the 24 selected treatments. The fitted models expressing the relationships between the microbial indicators and SOC content, their R^2 values, and the threshold limits of the adequacy classes are also presented in Fig. 5. First-order linear models were fitted for all microbial indicators except arylsulfatase, for which a quadratic model was fitted. As observed in Fig. 4, increased SOC levels also corresponded to increased levels of MBC, basal respiration, and soil enzyme activities, indicating that the availability of SOC influences biomass and soil microorganism activity. This relationship may be attributed to the physical protection within aggregates promoted by the SOC and its role as a source of C, energy, and nutrients for the microbial communities (Dick and Burns, 2011).

Considering that it is easier to assess the SOC content of a given area than its RCY, a second strategy was outlined to derive an interpretative table for the microbial indicators that involved SOC. Based on the mathematical relationship between the RCY and SOC (Fig. 2), a critical level for SOC of 18.2 g kg^{-1} soil (equivalent to 80% of the RCY) was calculated. This critical level was similar to the value reported by Sousa and Lobato (2004) for clayey Cerrado Oxisols at a 0- to 20-cm depth. The interpretative classes for SOC content as a function of the RCY were also calculated based on the mathematical relationship between the RCY and SOC as follows: low: ≤15.2 g kg^{-1}; moderate: 15.3 to 18.2 g kg^{-1}; and adequate: >18.2 g kg^{-1}. These three classes were equivalent

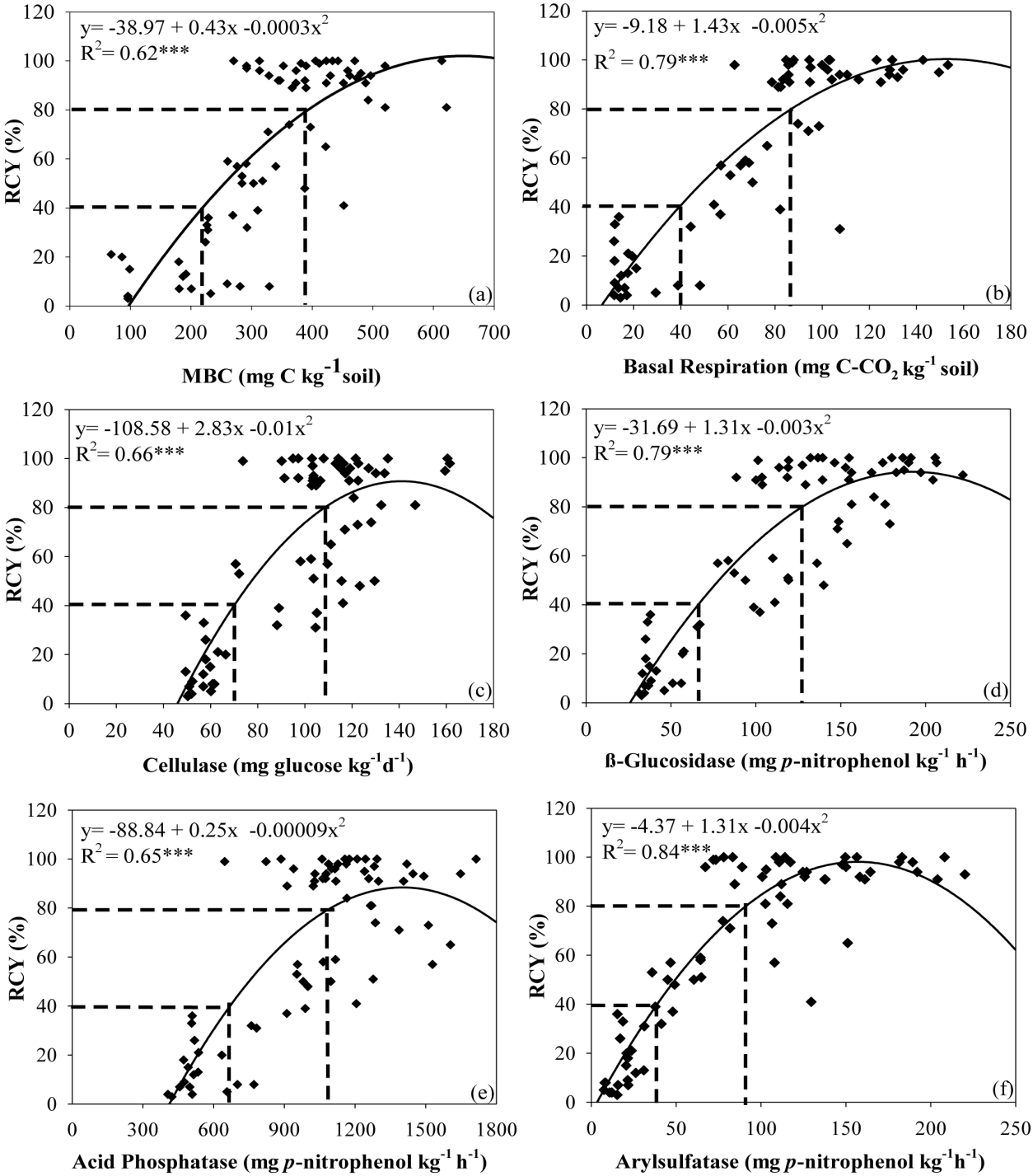

Fig. 4. Relationships among relative cumulative yield (RCY) and (a) microbial biomass C (MBC), (b) basal respiration, (c) cellulase, (d) β-glucosidase, (e) acid phosphatase, and (f) arylsulfatase. The data points represent the three field replicates of the 24 selected treatments. Dashed lines represent the limits of the interpretative classes: ≤40% RCY: low, 41 to 80% RCY: moderate and > 80% RCY: adequate. ***Significant at *P* < 0.001.

Table 4. Interpretative classes for microbial indicators in a clayey Red Latosol of the Cerrado region (0–10-cm depth) as a function of the relative cumulative yield (RCY).

Microbial indicator	Interpretative class as a function of RCY†		
	Low	Moderate	Adequate
Microbial biomass C, mg C kg^{-1} soil	≤215	216–375	>375
Basal respiration, mg C kg^{-1} soil	≤40	41–90	>90
Cellulase, mg glucose kg^{-1} soil d^{-1}	≤70	71–105	>105
β-Glucosidase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤65	66–115	>115
Acid phosphatase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤680	681–1160	>1160
Arylsulfatase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤40	41–90	>90

† Interpretative classes are: ≤40% RCY: low; 41–80% RCY: moderate; and >80% RCY: adequate.

to ≤40, 41 to 80, and >80% of the RCY, respectively. Based on the SOC contents that represented the upper and lower limits of each interpretative class, the corresponding values were calculated (using the equations shown in Fig. 5) and are displayed graphically for each microbial indicator (Fig. 5). The values of the interpretative classes

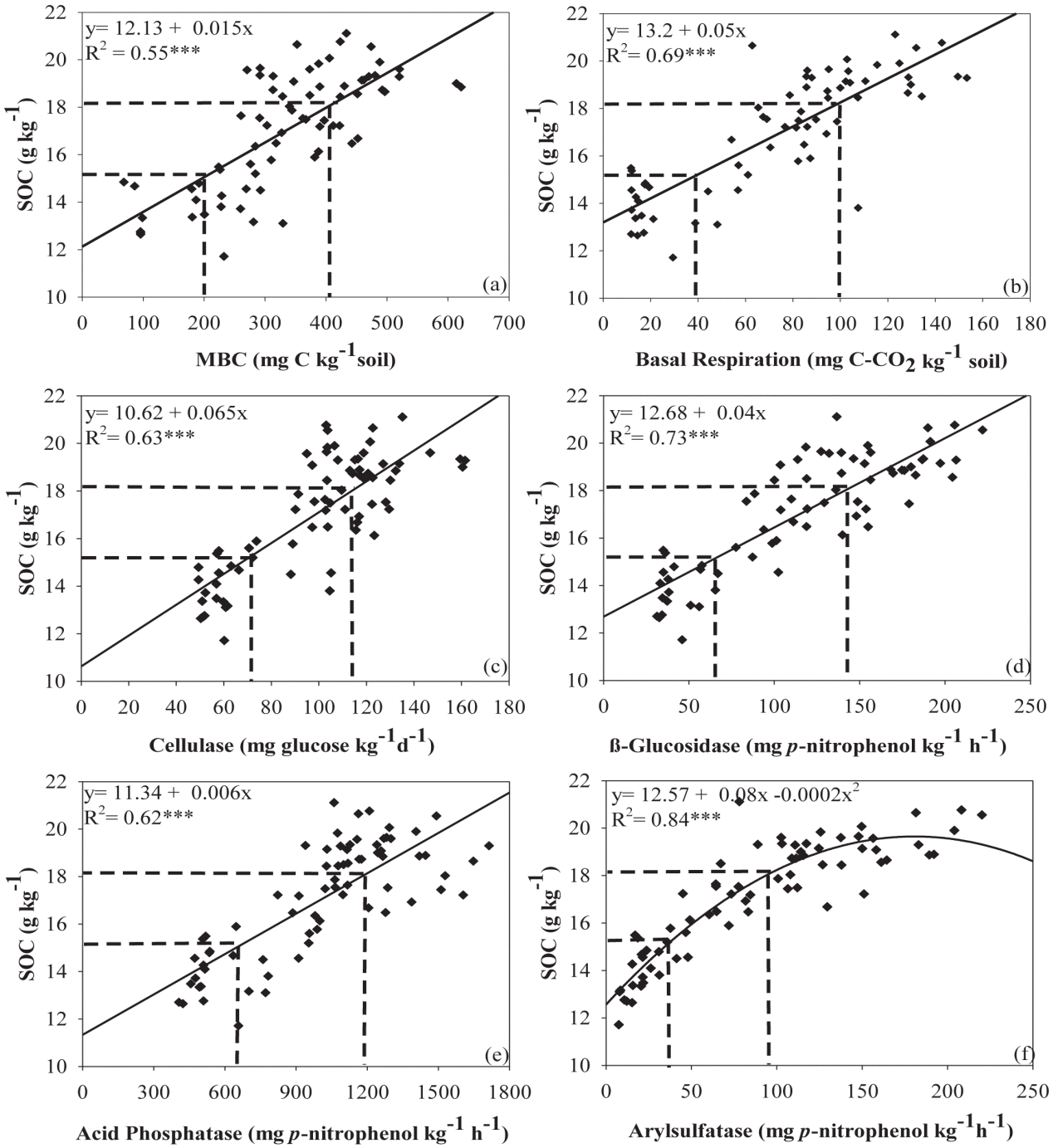

Fig. 5. Relationships among SOC and (a) microbial biomass C (MBC), (b) basal respiration, (c) cellulase, (d) β-glucosidase, (e) acid phosphatase, and (f) arylsulfatase. The data points represent the three field replicates of the 24 selected treatments. Dashed lines represent the limits of the interpretative classes for SOC: ≤15.2 g kg^{-1} soil: low; 15.3 to 18.2 g kg^{-1}soil: moderate; and >18.2 g kg^{-1} soil: adequate. * Significant at $P < 0.001$.**

for each microbial indicator as a function of SOC content are shown in Table 5.

Three approaches to derive the upper and lower limits for the SOC content of different New Zealand soil orders were also reported by Sparling et al. (2003). Approach 1 (statistical approach) considered the median value of the soil C in the long-term pastures listed in the New Zealand Soils National Database to be the

Table 5. Interpretative classes for microbial indicators in a clayey Red Latosol of the Cerrado region (0- to 10-cm depth) as a function of the soil organic C (SOC) content.

Microbial indicator	Interpretative classes as a function of SOC†		
	Low	Moderate	Adequate
Microbial biomass C, mg C kg^{-1} soil	≤205	206–405	>405
Basal respiration, mg C kg^{-1} soil	≤40	41–100	>100
Cellulase, mg glucose kg^{-1} soil d^{-1}	≤70	71–115	>115
β-Glucosidase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤60	61–140	>140
Acid phosphatase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤640	641–1150	>1150
Arylsulfatase, mg *p*-nitrophenol kg^{-1} soil h^{-1}	≤35	36–90	>90

† Interpretative classes for SOC are: ≤15.2 g kg^{-1}: low; 15.3–18.2 g kg^{-1}: moderate; and >18.2 g kg^{-1}: adequate.

upper limit (optimum C content) and the C content relative to the lower quartile of this same database as the lower limit. Approach 2 used the Century model to predict the maximum C contents in the long-term pastures, whereas Approach 3 was based on an expert opinion panel that defined the desirable C contents for the different soils based on production and environmental criteria. Although a good agreement was observed between Approaches 1 and 2, they considered that the statistical and expert opinion approaches were less defensible in setting lower limits for desirable C contents.

A comparison between Tables 4 (interpretation of the microbial indicators as a function of the RCY) and 5 (interpretation of the microbial indicators as a function of the SOC content) shows a good agreement between the two interpretations. This result was expected due to the fairly linear relationship between the RCY and SOC content (Fig. 2). Small differences between the lower and upper limits of the interpretative classes obtained using either the RCY or SOC were observed for all of the microbial indicators. It is important to emphasize that for the basis of our calculations, we chose values corresponding to $\leq$40% (low), 41 to 80% (moderate), and >80% (adequate) of both the RCY and SOC content as the limits of our interpretative classes and that we are aware that other limits could be applied.

The two interpretation tables presented are a first approximation that will probably be adjusted based on the results of similar studies in the future. The thresholds presented here will probably differ for other soil types, soil textures, or land uses (pastures, perennials, planted forests). For those parameters for which we could find references in the literature (MBC and β-glucosidase activity), however, it is interesting to note that the adequacy values obtained in Tables 4 and 5 for MBC (375 and 405 mg C kg^{-1} soil, respectively) and β-glucosidase activity (115 and 140 mg *p*-nitrophenol kg^{-1} soil h^{-1}, respectively) were in a reasonably good agreement with the appropriate levels already published. For the Natural Resource Inventory pilot project and for the Iowa case study data set (mostly Mollisols, Alfisols, and Entisols), Andrews et al. (2004) reported a MBC appropriate level of 400 mg C kg^{-1} soil. In the curves proposed by Stott et al. (2010) for β-glucosidase activity in a silty clay Hapludox under high-temperature and high-precipitation conditions, the appropriate activity level (equivalent to a β-glucosidase score of 0.99) was 160 mg *p*-nitrophenol kg^{-1} soil h^{-1}. Although this value differs from our reference level based on 80% RCY (115 mg *p*-nitrophenol kg^{-1} soil h^{-1}), it is similar to the activity value corresponding to 100% RCY, 157 mg *p*-nitrophenol kg^{-1} soil h^{-1} calculated using the equation displayed in Fig. 4D.

In addition to assisting in the interpretation of microbial indicators independent of a comparative control or treatment, these tables can also be useful in terms of establishing lower threshold limits, baseline values, and upper threshold limits for standardized scoring functions to assess the changes in soil quality using soil quality indexes (e.g., Karlen and Stott, 1994; Hussain et al., 1999). In this regard, the β-glucosidase activity values reported in the present work corresponding to 80 and 40% RCY, 115 and 65 mg *p*-nitrophenol kg^{-1} soil h^{-1}, respectively, were equivalent to β-glucosidase scores of 0.854 and 0.319, calculated using the algorithm proposed by Stott et al. (2010) and assuming a 4-4-1 condition (e.g., a clayey soil, with low potential for sequestering SOC, and with climate designation high temperature–high precipitation). Considering that scores of 0.8 and 0.3 in the Soil Management Assessment Framework (Stott et al., 2011) mean that a soil indicator is at 80 and 30% of the optimum or maximum value, respectively, these results show a good agreement between our strategy based on RCY values and the Soil Management Assessment Framework scoring curves. On a more-is-better sigmoidal curve, a score of 1 means that its plateau was reached, i.e., above this value, the parameter being evaluated is no longer a limiting factor to pertinent soil functions and processes (Andrews et al., 2004).

We believe that the use of interpretative classes based on crop yields, proposed in this study, to evaluate the soil quality of agricultural soils, seems more adequate and realistic than the use of reference soils under native vegetation with minimum anthropogenic impacts. The biological functioning of native Cerrado soils, for instance, has some peculiarities (Mendes et al., 2012) that, if not well understood, can lead to erroneous interpretations of the biological indicators. For example, in Table 6, the native Cerrado areas (average SOC content of 19.7 g C kg^{-1}) present consistently lower β-glucosidase activities (average of 57 mg *p*-nitrophenol kg^{-1} soil h^{-1}) than the cultivated soils with similar SOC contents (Fig. 5D). This observation, which could be considered an anomaly (Stott et al., 2010), is related to the quality and quantity of the returned plant residues, which are more complex in these native areas than those commonly found in agricultural fields (Peixoto et al., 2010) and result in decreased activities of β-glucosidase, a soil enzyme that acts in the final rate-limiting step in cellulose degradation (Tabatabai, 1994).

The basal soil respiration in these native Cerrado areas (Table 6) also follows the same rationale. In addition to the fact that the respiration rates tend to be lower in natural ecosystems (because soil microbial populations are in equilibrium), due to the higher complexity of plant residues found in native Cerrado areas, the accumulation of readily mineralizable C is smaller than in the cultivated areas. This situation results in lower levels of basal respiration in the native soils (average of 60 mg CO_2–C kg^{-1} soil) compared with the cultivated soils with similar SOC contents (Fig. 5B).

Conversely, the use of interpretative classes based on the relationships of the microbial indicators with the RCY and SOC content (Tables 4 and 5) shows strong evidence that supports the idea that the equilibrium MBC levels found in the native areas (average of 667 mg C kg^{-1} soil; Table 6) may not be attainable targets in agricultural Cerrado soils, even under the best management practices. In short, the use of the native Cerrado area as the soil quality reference criterion would result in high-

Table 6. Microbial indicators and soil organic C (SOC) in three native Cerrado sites and their averages. Samples were collected at the 0- to 10-cm depth.

Site	Microbial biomass C	Basal respiration	Cellulase	β-Glucosidase	Acid phosphatase	Arylsulfatase	SOC
	—— mg C kg^{-1} soil ——		mg glucose kg^{-1} soil d^{-1}	—— mg *p*-nitrophenol kg^{-1} soil h^{-1} ——			g kg^{-1}
Cerrado I	620 ± 45†	71 ± 4	114 ± 2	68 ± 3	1298 ± 41	60 ± 6	19.9
Cerrado II	632 ± 51	69 ± 3	121 ± 8	52 ± 1	1741 ± 173	53 ± 2	18.0
Cerrado III	750 ± 74	41 ± 9	127 ± 9	51 ± 2	1450 ± 129	70 ± 2	21.1
Avg.	667	60	121	57	1497	61	19.7

† Means ± standard errors, $n = 3$.

quality agricultural soils being penalized for having MBC levels inferior to those found in the native area.

Although the microbiological soil database is rather incipient in Brazil, particularly for soil enzymes, it has increased during the past 10 yr. The approach evaluated in the present study can be a starting point for future studies, and the interpretation tables may help soil scientists setting up a minimum data set of microbial indicators for their studies of soil quality. In this regard and as already reported by other researchers (Miller and Dick, 1995; Bandick and Dick, 1999; Stott et al., 2010), we also believe that soil enzyme measurements have great potential as soil quality indicators under tropical conditions due to their sensitivity, ease of measurement, and low cost.

CONCLUSIONS

We reported a site-specific interpretative framework for individual microbial indicators based on the principles of soil nutrient calibration tests. The relationships observed between the six soil microbial indicators with the RCY and SOC content showed that highly productive soils also presented high levels of microbial biomass, respiration, and activity levels of the soil enzymes cellulase, β-glucosidase, arylsulfatase, and acid phosphatase. Based on the mathematical expressions of these relationships, target ranges for each soil microbial indicator as a function of RCY and SOC content were established (≤40%: low; 41—80%: moderate; and >80%: adequate), and interpretative tables were generated. The critical levels equivalent to 80% of the RCY for MBC, basal respiration, cellulase, β-glucosidase, acid phosphatase, and arylsulfatase were: 375 mg C kg^{-1} soil, 90 mg CO_2–C kg^{-1} soil, 105 mg glucose kg^{-1} soil d^{-1}, 115 mg *p*-nitrophenol kg^{-1} soil h^{-1}, 1160 mg *p*-nitrophenol kg^{-1} soil h^{-1}, and 90 mg *p*-nitrophenol kg^{-1} soil h^{-1}, respectively. Similar results were obtained when SOC was used as the interpretation criterion. Considering that it is easier to assess the SOC content of a given area than it is to assess the RCY, the interpretation strategy based on the SOC has more practical advantages.

The development of this interpretative framework reinforces the strategic importance of long-term field experiments, whereby it is possible to establish positive and negative effects on the overall soil quality by implementing different land uses.

The interpretation tables provided in this study establish, for the first time, reference values for the soil microbial indicators based on crop yields and constitute a first approximation. Their applicability to other soil types, regions, and land uses must be evaluated.

ACKNOWLEDGMENTS

We thank Clodoaldo A. de Sousa, Lucas F.L.S. Rolim, Osmar Teago Oliveira, and Valmir V. de Sousa for their assistance during this study. We also thank the two anonymous reviewers for their constructive comments. This work was partially financed by the CNPq (National Council for Scientific and Technological Development) Edital de Redes REPENSA (562433/2010-4) and FAPDF (Research Support Foundation of the Federal District) Edital PRONEX. I.C. Mendes acknowledges a research fellowship from the CNPq.

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