Soil microbial response to glucose and phosphorus addition under agricultural systems in the Brazilian Cerrado

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ABSTRACT

Conventional tillage (CT) and no-tillage (NT) management systems alter soil nutrient availability and consequently modify soil microbial response to nutrient additions such as carbon (C) and phosphorus (P). The objective of this study is to evaluate microbial response to the addition of C (glucose) and P (Na$_2$HPO$_4$.7H$_2$O) under CT and NT in the brazilian Cerrado. In response to glucose addition, the NT system yielded higher microbial respiration rates and glucose consumption than the CT system. The best microbial response to C addition was after 0 - 12 h incubation in NT and 0 - 24 h in CT. The addition of P produced higher demand under CT than NT. After incubation, biochemical indicators such as microbial respiration, glucose consumption, dehydrogenase activity and metabolic yield confirmed the higher glucose demands under NT and higher phosphorus demands under CT. These results demonstrate that C and P addition alter significantly the microbial response, suggesting that soil microorganisms present nutrient differential demands between CT and NT management systems.

Key words: dehydrogenase, glucose assay, Michaelis-Menten equation, nutrient demands, incubation.

INTRODUCTION

Evaluation of soil microbial activity is of great importance in measuring the functional state of terrestrial ecosystems due to the fundamental role microorganisms play in energy flow, nutrient cycling and organic matter transformation. However, soil microbial activity is regulated by a 30 number of abiotic and biotic factors (Atlas and Bartha 1997, Han et al. 2007, Martins et al. 2011). Abiotic factors such as nutrient levels, temperature and moisture can typically be used to evaluate the potential responses of the microbial community. Evaluation of microbial activity by the amount of CO$_2$ produced has been used as an indicator of soil metabolic response,

partly due to the simplicity of associated assays (Stotzky 1965, Hill et al. 2008). Therefore, metabolic response may be used as an additional attribute for predicting the impact of agricultural practices on terrestrial ecosystem function.

In tropical soils, there is evidence that soil microbial respiration is strongly regulated by carbon and phosphorus availability (Cleveland et al. 2002, Ilstedt et al. 2003, Ehlers et al. 2010). Due to their low soil content, carbon sources, such as glucose, sucrose and amino acids are usually the limiting factors of microbial activity (Cleveland et al. 2002, Hill et al. 2008). Under natural conditions, soluble carbon availability results from the decomposition of plant material which is affected by daily and seasonal fluctuations such as temperature and moisture

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(Nicolardot et al. 1994, Kirschbaum 2006, Vanhala et al. 2007, Van Meeteren et al. 2007). However, glucose addition to the soil has been utilized as a strategy for measuring the respiratory response of the soil microbial community (Shen and Bartha 1996, Chotte et al. 1998, Blagodatskaya et al. 2007, Hill et al. 2008). Studies have also demonstrated that soil phosphorus (P) availability regulates microbial activity due to microorganism demand for this nutrient (Cleveland et al. 2002, Ilstedt et al. 2006, Ferreira et al. 2008). In addition, there is a correlation between P content in soil solutions and other biochemical processes such as decomposition and mineralization of soil organic matter (Chotte et al. 1998, Saggar et al. 1998, Cleveland et al. 2002) and high sesquioxide content in older soils (Ilstedt et al. 2003, Gnankambary et al. 2008).

Many soil biochemical characteristics are frequently measured by assays under controlled conditions. For example, assays can be performed to quantify soil microbial response to environmental disturbance (Atlas and Bartha 1997) and thereby reveal the factors regulating microbial respiration (Raich and Tufekcioglu 2000) and establish the efficiency of substrate mineralization (e.g., glucose) by soil microorganisms (Shen and Bartha 1996 Ananyeva et al. 2008, Hill et al. 2008). Soil biochemical properties are important indicators in the evaluation of land-use (Chotte et al. 1998, Ananyeva et al. 2008, Hill et al. 2008, Trasar-Cepeda et al. 2008), including tillage systems.

Cerrado vegetation covers an area of about 204 million ha (approximately 22% of the Brazilian territory) and is considered to be the country's second largest biome. Cerrado soils are used for a variety of agricultural activities, including grain production, stock raising and forestry. The predominant soil types in the region are Oxisols, highly weathered, deep, porous and have clay content above 15%. They also generally hold small nutrient reserves for plant growth. Due to these characteristics, Oxisols have low cationic exchange capacity and high anionic adsorption, especially regarding phosphates.

In Brazil, few studies have been conducted on soil microbial activity in Cerrado regions under conventional tillage and no-tillage systems (Ferreira et al. 2008). Thus, the objective of this work was to evaluate soil microbial response to glucose and phosphorus addition under conventional tillage and no-tillage systems in the Brazilian Cerrado.

MATERIALS AND METHODS

The study was performed on soil samples of Cerrado typic acrustox from areas under conventional tillage (CT) and no-tillage (NT). The experiment was conducted in southern Brazil at the Agraria Science Institute of the Universidade Federal de Uberlândia. Annual precipitation in this region ranges from 1,500 to 1,700 mm, and annual temperatures range from 12 to 34°C, with a mean annual temperature of 22.7°C. In the summer of 2008, soil samples of 600 cm^2 (20 cm x 30 cm) were taken down to a depth of 10 cm. For each field sampling, four samples were combined to make composite samples which were transported to the laboratory in isothermic bags (4°C). A portion of each sample was sieved (< 4 mm), and moisture content was determined after 48h at 105°C. The remaining soil was stored at 4°C for a maximum of two weeks until the assay was analyzed. Additional physiochemical characteristics of the air-dried soil were analyzed (Table I). Microbial biomass carbon (Vance et al. 1987) and glucose content (Ferreira et al. 2008) were determined using moist soil, and the results were adjusted to the dry weight of the soil.

TABLE I
Some background soil characteristics.

Soil characteristics	CT	NT
Carbon organic (g.kg^{-1})	11.1	45.2
Available phosphorus (mg.kg^{-1})	3.6	40.1
Available potassium (mg.kg^{-1})	11.0	335
pH in water (1:2)	5.6	6.4
MBC (mg.kg^{-1})	184	450
Total glucose (ug.kg^{-1})	15.3	6.23
Dehydrogenase (ug INTF.g^{-1}.soil h^{-1})	15.2	21.3

Soil microbial respiration assays were performed with glucose and phosphorus ($Na_2HPO_4.7H_2O$). Portions of moist soil (50 g) were placed in glass bottles (300 mL) containing glucose (0, 50, 100, 200, 400, 600, 800, 1,200 and 2,000 mg glu.kg^{-1} dry soil) and phosphorus nutrients (0, 50, 100, 200, 300, 400 and 600 mg P.kg^{-1} dry soil). Four replicates were performed for each experiment. Distilled water was used to adjust soil moisture content to 60% of water-holding capacity. The bottles were incubated at room temperature which ranged from 21° to 23°C and averaged 22.3°C. Microbial respiration response to glucose addition was measured after incubation periods of 0 -12h, 0 - 24h, 12 - 24h and 24 - 48h. Respiration response to phosphorus addition was evaluated after 3 and 7 days of incubation at room temperature.

Microbial respiration was determined by measuring released CO_2 (Stotzky 1965). This assay was carried out in glass bottles (300 mL) containing soil and plastic cups with 5 mL of sodium hydroxide (1 mol L^{-1}). The C-CO_2 collected in the alkaline solution was determined by titration of the residual NaOH with chloride acid (0.25 mol.L^{-1}) after the addition of 2.5 mL of $BaCl_2.2H_2O$ (1 mol.L^{-1}) and phenolphthalein indicator. The C-CO_2 produced was expressed in mg C-CO_2.kg^{-1} dry soil.

Soil samples (10 g) were transferred to Falcon bottles (50 mL), and glucose was added as described above. An aliquot of sugar in solution was used for each treatment. Soil moisture was adjusted to 60% of water-holding capacity with distilled water. The bottles were incubated at room temperature for 0, 6, 12, 18 and 24 h. After incubation, the glucose content of each treatment was extracted as described in a previous study (Ferreira et al. 2008). The bottles were transferred to a microwave oven and exposed to electromagnetic irradiation for 1 min (1.62 10^5 J). Then, 15 mL of NaCl (0.5%) was added and the bottles were agitated for 20 min at 70°C. The soil solution was filtered (Whatman n#. 40 paper filter) and centrifuged at 8,000 x g for 10 min. An aliquot (25 -250 uL) of the supernatant was removed to determine the amount of glucose remaining in the soil.

Glucose content was determined by the glucose oxidase-peroxidase method, following the recommendations of the kit manufacturer (Sigma, USA). A standard curve was generated with known amounts of glucose (0, 2, 4, 8, 16 and 32 ug.mL^{-1}). The absorbance of the samples was measured with a cuvette in a spectrophotometer at 500 nm.

Dehydrogenase activity was measured by reduction of 2-p-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride (INT) to iodonitrophenyl formazan (INTF) with modification to the method reported by Von Mersi and Schinner (1991). Portions of moist soil (1 g) were transferred to Falcon bottles (50 mL) containing 1.0 mL Tris (1 mol.L^{-1}, pH 7.0) and 1 mL INT (10 mmol.L^{-1} in 2% N,N-dimetilphormamide). The mixture was incubated at 37°C for 24 h. After incubation, INTF was extracted using 10 mL of N,N-dimetilphormamide: ethanol (1:1) and then agitated and rested for 20 min. An aliquot of 2 mL was centrifuged at 6,000 x g for 5 min and the amount of INTF in the supernatant was measured by spectrophometry at 464 nm. An INTF standard curve was used to determine dehydrogenase activity in the soil samples.

The induced response of microbial activity was performed under assay conditions with the addition of carbon (1.6 g C.kg^{-1} dry soil) , nitrogen (200 mg N.kg^{-1} dry soil), and phosphorus (400 mg P.kg^{-1} dry soil) to the soil. Carbon, nitrogen and phosphorus sources were glucose, ammonium nitrate (NH_4NO_3) and sodium phosphate ($Na_2HPO_4.7H_2O$), respectively. The nutrient treatments were: (1) control, (2) C, (3) P, (4) C + P, (5) N, and (6) C + N + P. Metabolic yield (Y) of the soil microbes was obtained from the ratio of substrate carbon oxidation to CO_2 and carbon assimilation by the soil microbial community (Shen and Bartha 1996) during the 24h incubation period.

Results were submitted to factorial ANOVA and the differences among treatment means were tested at a 5 % probability level. Regression analysis of the Michaelis-Menten equation was performed to determine microbial response to nutrient additions, where

$$MR = (MR_{max}N)/(k_m + N).$$

MR is the microbial respiration rate (mgC-CO_2.kg^{-1}) per day or hour, MR_{max} is the maximum rate of microbial respiration, N is nutrient added (mg N.kg^{-1} dry soil, either glucose or phosphorus) and k_m is the Michaelis-Menten constant (mg N.kg^{-1} dry soil). The kinetic response parameters were limited to a probability level of 5%.

RESULTS

The highest microbial respiration response to glucose addition was observed in soil samples incubated for 24 h (Fig. 1B). However, both soils showed a differential response when the Michaelis-Menten equation was applied. In NT, best fit was observed for the period of 0 - 12 h (Fig. 1A), with values of MR_{max} = 130 mg C-CO_2.h^{-1} kg^{-1} dry soil and km = 693 mg glu.kg^{-1} dry soil ($R^2_{ajusted}$ = 0.981). In CT, the values were MR_{max} = 36.5 mg C-CO_2.h^{-1}.kg^{-1} dry soil and km = 178 mg glu.kg^{-1}.dry soil ($R^2_{ajusted}$ = 0.961) for the same incubation period. In CT, the best fit was observed for the incubation period of 0 - 24 h (Fig. 1B) with values of MR_{max} = 126 mg C-CO_2.h^{-1}.kg^{-1}.dry soil and km = 845 mg glu.kg^{-1}.
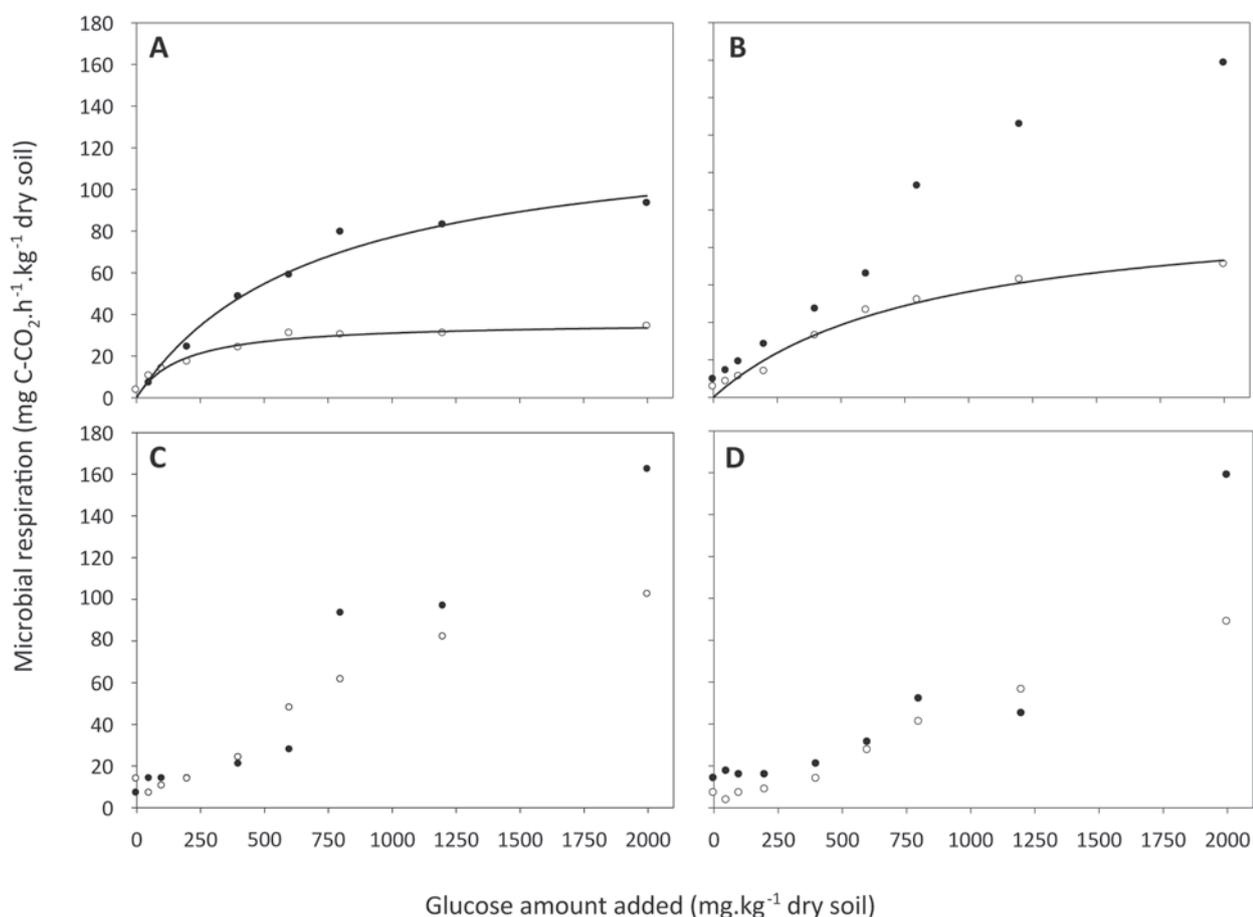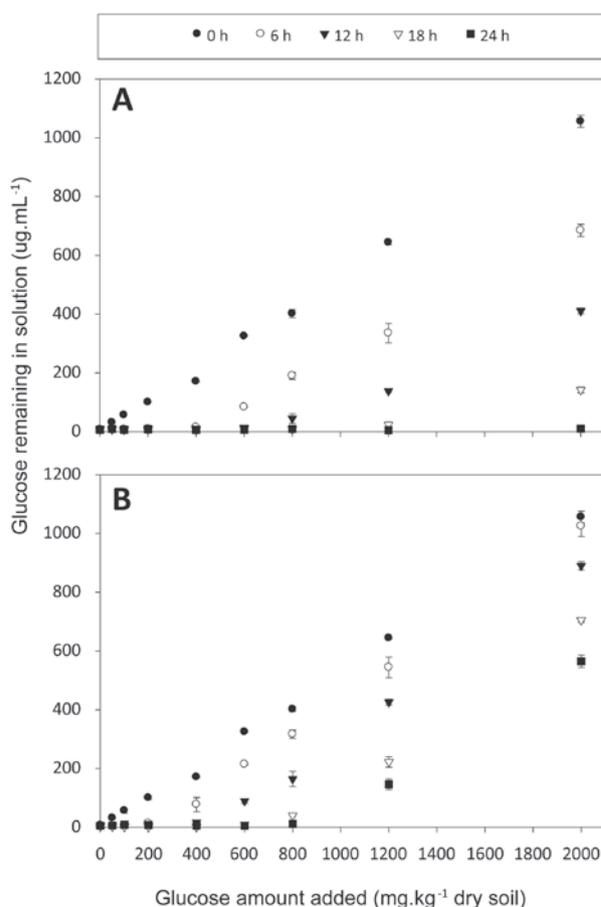

Fig. 1 - Microbial respiration of soils in response to addition of glucose and incubation period under NT (●) and CT (○) systems. **A**, 0-12h of incubation. **B**, 0-24h of incubation. **C**, 12-24h of incubation. **D**, 24-48h of incubation. Data represent the mean of four assays under conditions of room temperature. The lines in A and B show the best fit to the Michaelis-Menten equation to a probability level of 5% (n=4).

dry soil ($R^2_{ajusted}$ = 0.978). Regression analysis did not yield a good fit for the other incubation periods when the Michaelis-Menten equation was applied at 5 % probability (Fig. 1C and 1D).

Glucose consumption was higher in NT than in CT for all assay periods (Fig. 2). After 24 h of incubation, glucose had been consumed in all NT treatments, including the treatment with 2,000 mg glucose kg^{-1} dry soil. Remaining glucose was higher for all CT assays than it was for NT.

Figure 3 shows the kinetic parameters obtained from the Michaelis-Menten equation for microbial respiration in both soils with added phosphorus (Fig. 3). Results showed high regression coefficients

for both soils (with R^2 higher in CT). MR_{max} in CT was about 2.1 times higher than in NT; however, k_m of CT was about 1.5 times lower. The results clearly revealed that phosphorus additions yielded significant microbial respiration gains in NT (>MR_{max}), while significant P demand was observed in CT under laboratory assay conditions (< k_m).
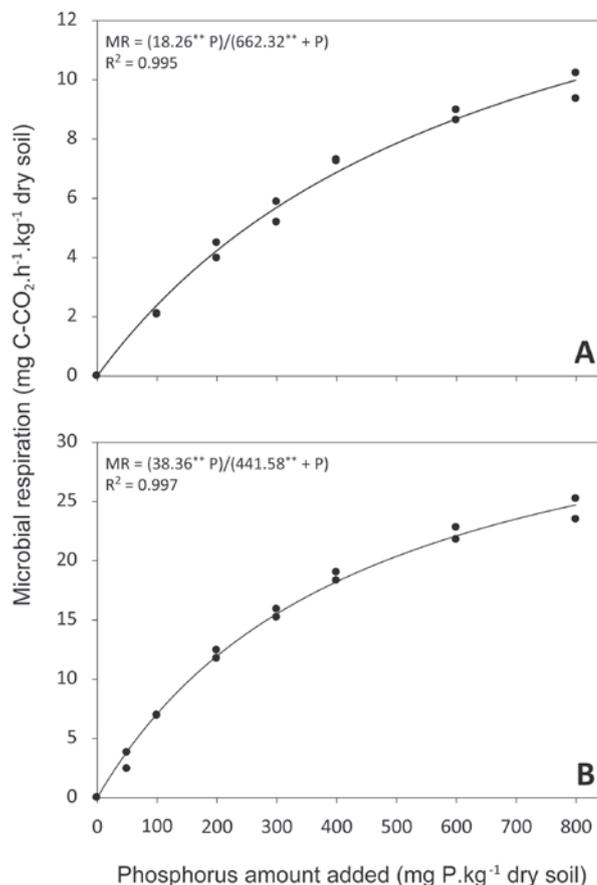
Fig. 3 - Microbial respiration in response to phosphorus addition in soils. The soil samples are represented with NT (**A**) and CT (**B**). The signs (**) show that kinetic parameters to the Michaelis- Menten are significantly fitted at a probability level of 5% (N=2).

Fig. 2 - Glucose remaining in soil solution extracted with NaCl (0.5%) under incubation conditions for 0, 6, 12, 18 and 24h at room temperature. Pattern the glucose consumption in NT (**A**) and CT (**B**). The bars show the confidence intervals to 95% probability (n=3).

Microbial respiration, dehydrogenase activity and metabolic yield after the addition of glucose (G), phosphorus (P) and nitrogen (N) are shown in Table II. Except for dehydrogenase activity in CT, glucose addition to both soils increased metabolic response compared to the control. There was no metabolic response (microbial respiration and

dehydrogenase activity) for soils in the treatments with P and N after 24 h of incubation, when analyzed separately. However, metabolic response increased in the CT (G + P treatment), whereas this increase was not observed in NT. In the treatment with G + P + N, increases in microbial respiration and dehydrogenase activity were observed.

The results (Table II) also show that metabolic yield increased when phosphorus was applied, with substantial increases in CT. Compared to the P treatment, N addition did not alter metabolic yield, although there was an additional increase when N was applied together with glucose and phosphorus (treatment G + P + N).

TABLE II
Microbial responses to nutrient addition under conventional tillage (CT) and no-tillage (NT) systems.

Treatment	Microbial respiration (mg C-CO$_2$.kg^{-1} dry soil)		Dehydrogenase (ug INTF.g^{-1} dry soil.h^{-1})		Metabolic yield (Y)	
	CT	NT	CT	NT	CT	NT
Control	17.4 d	19.0 c	16.8 c	18.7 c	--	--
G	98.8 b	270.0 b	19.0 c	25.0 b	0.077	0.225*
P	36.4 d	35.2 c	19.2 bc	19.8 c	--	--
G + P	169.0 c	269.0 b	22.6 b	26.5 b	0.230	0.296*
N	17.4 d	23.6 c	16.3 c	18.3 c	--	--
G + P + N	289.0 a	350.4 a	28.6 a	28.3 a	0.247	0.309*

Treatments with the same letter on columns no differ between them in relation the variables (microbial respiration and dehydrogenase), when applied the Tukey test at the 95 % probability.

* this sign shows that there are significant differences of Y between CT and NT in each treatment, when applied the orthogonal contrast test at the 95% probability.

DISCUSSION

In this study, an applicable approach for evaluating the soil microbial response of two tillage systems from a region in the Brazilian Cerrado was demonstrated. Changes in microbial respiration, glucose consumption and metabolic yield were observed in response to glucose and phosphorus additions. Some studies have reported on soil microbial response to glucose and phosphorus addition under laboratory assay conditions, providing insight into the nutritional demands of different soils (Saggar et al. 1998, Cleveland et al. 2002, Ilstedt and Singh 2005, Boddy et al. 2008, Schneckenberger et al. 2008).

In response to the addition of glucose, microbial respiration was consistently different between tillage systems (Fig. 1), revealing that NT had higher demand for soluble carbon as an energy source. This observation was also confirmed by sugar consumption during incubation (Fig. 2). Higher microbial respiration response in NT can also be related to the amount of available nutrients in this soil (Table I). This study showed that the fit of the Michaelis-Menten equation depended on incubation period, as observed in CT (Fig. 1B). For NT, fit was not determined for the 0 - 24 h incubation period due to the absence of significant kinetic parameters. The 0 - 12 h incubation period resulted in k_m that was 3.89 times higher for NT than it was for CT. This equation has been used to indicate the kinetic response to low molecular weight compound soil additions such as glucose and amino acids (Vinolas et al. 2001, van Hees et al. 2005, Boddy et al. 2008, Schneckenberger et al. 2008). Here, it was demonstrated that the Michaelis-Menten equation can be of great importance in evaluating soil microorganism response to glucose addition under different agricultural systems.

Measuring glucose content in the soil using the glucose oxidase-peroxidase method was quite suitable. About 94 % of the glucose applied to the soil (control) was recovered in the extraction solution (0.5 % sodium chloride). Some studies, such as Boddy et al. (2008) and Nambu et al. (2008), have determined soil glucose content by this method, but have not provided details on their extraction and determination methods. Finding soil glucose content by this method could be important for studies evaluating the utilization efficiency of sugar as a carbon and energy source for soil microorganisms. In addition, glucose assays have been used to evaluate soluble carbon (C) mineralization models (Shen and Bartha 1996, van Hees et al. 2005), organic C turnover (Chotte et al. 1998, Boddy et al. 2008), and soil microbial biomass C estimation (Anderson and Domsch 1978).

This study demonstrated the microorganism response to the addition of P to the soil (Fig. 3A and 3B). The effect of phosphorus addition on microbial response was substantial under CT when analyzed with the Michaelis-Menten regression equation, demonstrating that microbial demand for available phosphorus is high under CT. For many old soils in tropical ecosystems, phosphorus is usually believed to be the most limiting nutrient due to high nutritional demand of plants and microorganisms (Cleveland et al. 2002). In many terrestrial ecosystems, high P demand and its low availability in soils provide solid arguments that this nutrient acts as a metabolic regulator of microbial activity (Crews et al. 1995, Cleveland et al. 2002, Ilstedt et al. 2006).

In the final analysis of the assays, it was confirmed that differences exist between soils regarding metabolic response to nutrient additions (Table II). In the 24 h incubation assay, P addition produced greater metabolic response increases for CT. However, for NT, metabolic response increased after glucose addition. N addition did not yield any metabolic response increases. However, there were additional increases in metabolic response when N was added together with G and P. Addition of P increased soil metabolic yield, suggesting that there was a greater efficiency in glucose utilization and showing greater capacity of microorganisms to mineralize the carbon soluble. These results also suggest soil microbial activity is strongly regulated P in the soil, but the microorganism response depends on land-use systems. However, more studies should be undertaken to elucidate the mechanisms involved in the action of P in soil, especially in tropical ecosystems.

This study also showed that microbial respiration, dehydrogenase activity and metabolic yield can be used as indicators to measure soil metabolic responses to nutrient additions. Particularly, dehydrogenase enzyme has been linked to greater carbon availability for microorganisms given that its activity is directly linked to intracellular transformations of organic compounds (Pajares et al. 2011) with consequent implications for soil processes such as the mineralization of organic matter (Pankhurst et al. 1998, Lagomarsino et al. 2009).

Quantifying and understanding the dependence of soil microbial activity on nutrients remains a key focus for investigating soil ecosystem function. In particular, the quest for reference values of soil metabolism associated with nutrient availability has been ongoing due to the fundamental role nutrients play in regulating plant and soil microorganism growth (Coody et al. 1986, Kouno et al. 2002). In Brazil and specifically in the Cerrado, little information regarding microbial response has been generated (Ferreira et al. 2008) which suggests a need for more studies to increase knowledge of biological transformations in the soil.

CONCLUSIONS

In response to glucose addition, microbial respiration rates (C-CO$_2$ efflux) were higher with NT than with CT in typic acrustox of the Cerrado. A similar response was observed with glucose consumption under laboratory assay conditions,

suggesting that NT systems require more carbon than CT systems. Microbial response to P addition was higher in the CT system than in the NT system, implying that phosphorus played a greater role in soil microbial respiration in CT than in NT. Biochemical indicators such as dehydrogenase activity, microbial respiration and metabolic yield confirmed these greater glucose demands in NT and phosphorus in CT after an incubation period of 24h under laboratory assay conditions.

ACKNOWLEDGMENTS

RESUMO

Os sistemas convencional (SC) e plantio direto (SPD) alteram a disponibilidade de nutrientes no solo e, consequentemente, modificam a resposta da microbiota do solo à adição de nutrientes tais como carbono (C) e fósforo (P). O objetivo deste estudo foi avaliar a resposta da microbiota do solo à adição de C (glicose) e P (Na$_2$HPO$_4$.7H$_2$O) sob os SC e SPD no Cerrado brasileiro. Em resposta à adição de glicose, o sistema SPD apresentou maior taxa de respiração microbiana e consumo de glicose do que o SC. A melhor resposta da microbiota à adição de carbono foi após 12 horas de incubação no SPD e 24 horas no SC. A adição de P produziu um maior efeito sob SC do que SPD. Após a incubação, os indicadores bioquímicos, como respiração microbiana, consumo de glicose, atividade de desidrogenase e rendimento metabólico, confirmaram a maior demanda de glicose em SPD e de P em SC. Esses resultados demonstraram que as adições de C e P alteram significativamente a resposta microbiana do solo, sugerindo que os micro-organismos do solo apresentam uma demanda diferencial de nutrientes entre os sistemas SC e SPD.

Palavras-chave: desidrogenase, ensaio de glicose, equação de Michaelis-Menten, demanda de nutrientes, incubação.

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