

Biofertilizers on soil microbial biomass and activity

Ademir S. F. de Araújo¹, José R.Oliveira¹, Raul M. Araújo², Regina L. F. Gomes¹

¹ Universidade Federal do Piauí, Centro de Ciências Agrárias, Campus da Socopo, Socopo, CEP 64000-000, Teresina-PI, Brasil. E-mail: ademir@ufpi.edu.br; asfaruaj@yahoo.com.br; rfgomes@ufpi.edu.br

² Universidade Federal de Pelotas, Campus Capão do Leão, Faculdade de Agronomia Eliseu Maciel, Centro, CEP 96010-900, Pelotas-RS, Brasil. E-mail: raulmatos@yahoo.com.br

ABSTRACT

The use of biofertilizers may improve soil microbial properties. However, according to the different composition of biofertilizers the effects may be different. The aim of this study was to evaluate the effect of different types of biofertilizers on soil microbial biomass and enzyme activities. We used three types of biofertilizers: Bio1, Bio2 and Bio3 and two additional treatments with chemical fertilization (NPK) and no fertilization (control). The biofertilizers presented different compositions and affected significantly the soil microbial properties. The application of all biofertilizers increased soil microbial biomass C (SMB-C) and soil respiration as compared with both controls. The highest and lowest values of SMB-C, soil respiration and soil enzymes were found for the biofertilizers Bio1 and Bio3, respectively. The application of biofertilizers increases the soil microbial biomass and activity. However, there are different responses of soil microbial biomass to the different compositions and qualities of biofertilizers. In this study, the biofertilizer Bio1 promoted the best response of soil microbial biomass and activity.

Key words: microorganisms, organic amendment, soil enzymes, soil quality

Biofertilizantes sobre a biomassa e atividade microbiana do solo

RESUMO

O uso de biofertilizantes pode melhorar as propriedades microbianas do solo. Entretanto, de acordo com as diferentes composições dos biofertilizantes os efeitos podem ser diferenciados. O objetivo deste estudo foi avaliar o efeito de diferentes tipos de biofertilizantes sobre a biomassa e a atividade microbiana do solo, razão por que foram utilizados tres tipos de biofertilizantes: Bio1, Bio2 e Bio3, além de dois tratamentos adicionais com e sem fertilização química. Os biofertilizantes apresentaram diferentes composições químicas, o que afetou as propriedades microbianas do solo. A aplicação dos biofertilizantes aumentou o C microbiano do solo (CBM) e a respiração comparada com ambos os controles. Os maiores e menores valores de CBM, respiração do solo e atividade de enzimas, foram encontrados com o Bio1 e Bio3, respectivamente. Os diferentes tipos de biofertilizantes afetam os microrganismos enquanto o uso do biofertilizante Bio1 promoveu a melhor resposta da biomassa e atividade microbiana do solo.

Palavras-chave: microrganismos adubação orgânica; Enzimas do solo; qualidade do solo

Introduction

The intensive use of soils has increased the loss of soil fertility and productivity, mainly because of decreasing soil organic matter. One of the alternative methods to maintain and improve the organic matter content and soil fertility is the use of biofertilizers (Chiconato et al., 2013). Biofertilizers are low cost, renewable sources of plant nutrients which supplement chemical fertilizers (Mohammadi & Sohrabi, 2012). These products are being strongly used due to the increasing emphasis on maintenance of soil health and decreasing in the environmental pollution and cut down on the use of chemicals in agriculture (Vessey, 2003). Currently, many different microbial biofertilizers are available for agricultural use and they enhance plant growth and yield and improve soil conditions, due to the addition of beneficial microbial inoculants to soil and by stimulation of soil microorganisms (El-Yazeid et al., 2007).

In Brazil, various types of biofertilizers are used regionally, prepared with animal and plant wastes, which are applied usually in the soil (Barros & Liberalino Filho, 2008). These biofertilizers present metabolites, such as enzymes, antibiotics, vitamins, phenols, and other compounds, such as esters and acids, which can influence the soil microbial biomass (Jbhiye et al. 2003). The soil microbial biomass, the living component of soil organic matter (SOM), is a sensitive indicator of soil changes and produces a large array of enzymes which play essential roles in various ecological processes, such as the decomposition of organic materials (Araujo et al., 2013; Silva et al., 2013). Also, soil enzymes control both the supply of nutrients to plants and microbial growth (Burns et al., 2013). Therefore, soil microbial biomass and enzyme activities may respond sensitively to application of biofertilizers since that these products present high organic matter content and microorganisms.

The number of studies investigating the effect of organic biofertilizers is increasing because of its importance on the sustainable agriculture and environmental quality. It is well known that biofertilizers increase plant yield and improve soil fertility (Vessey, 2003; El-Yazeid et al., 2007; Coimbra et al., 2013). However, there are few studies about the effect of biofertilizers on soil microbial biomass and enzyme activities. We hypothesized that (1) biofertilizers increase soil organic matter content and soil fertility, thus affecting soil microbial biomass, and (2) the responses of soil microbial biomass differ according the different types of biofertilizers. Therefore, we evaluated the effect of different types of biofertilizers on soil microbial biomass and enzyme activities.

Material and Methods

The experiment was performed under field conditions at the Agricultural Science Center, Teresina, Piauí State (05° 05' S;

42° 48' W, 75 m). The regional climate is dry tropical (Köppen), and it is characterized by two distinct seasons, a rainy summer and dry winter, with an annual average temperature of 30°C and a rainfall level of 1200 mm. The rainy season extends from January to April during which 90% of the total annual rainfall occurs. The soil is classified as a Fluvisol with the following composition at a 0–20 cm depth: clay, 10%; silt, 28% and sand, 62%.

In this study was evaluated three types of biofertilizers: Bio1, Bio2 and Bio3 (Table 1). Two additional treatments were added: chemical fertilization (NPK) and no fertilization (control). Thus, the experiment was running using five treatments: Bio1, Bio2, Bio3, NPK and control.

The biofertilizers were analysed according their composition at the Laboratory de Animal Nutrition from UFPI according to Embrapa (2009). The chemical properties of each biofertilizers are shown in Table 2.

Table 2. Chemical properties of the biofertilizers

	Bio1	Bio2	Bio3
Total C (%)	3.98	2.70	1.67
Total N (%)	0.40	0.15	0.19
P (g kg ⁻¹)	0.40	0.34	0.18
K (g kg ⁻¹)	5.40	2.30	2.20
Ca (g kg ⁻¹)	5.61	2.88	2.61
Mg (g kg ⁻¹)	1.84	0.95	0.70
S (g kg ⁻¹)	0.19	0.08	0.04

The experiment was a completely randomized design with four replications. The plots were marked out (20 m² each with 12 m² of area for the soil and plant sampling) and included rows spaced 1.0 m apart. The biofertilizers were applied at concentration of 10% and these applications were done at 7, 14, 21, 28 and 56 days after the sowing of *Capsicum*. The biofertilizers were spread uniformly on the soil surface. In the NPK treatment, it was applied 300 kg/ha of formulation 10-10-10 at the plant sowing.

Soil samples were collected, in each plot, at 10, 40, 60 and 100 days after plant sowign. In each plot, four samples were collected (0–20 cm), sieved and stored at 4°C prior analyses. Soil chemical properties (Table 3) were determined, at the beginng and at the end of experiment, according Tedesco et al. (1995). Soil pH was determined in a 1:2.5 soil/water extract. Exchangeable Ca was determined using extraction with 1 M KCl. Available P and exchangeable K were extracted using Mehlich-1 extraction method and determined by colorimetry and photometry, respectively. Soil organic matter was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans & Bremmer, 1998).

Before the microbial analysis, the soil was pre-incubated (with a 100 ml jar of soda lime and bottles containing deionized water) in the sealed plastics for 7 days at moisture of 60–70%

Table 1. Composition of the biofertilizer

Biofertilizer	Composition ^a
Bio1	24 L fresh cattle manure; 1600 g ZnSO ₄ , 1600 g MgSO ₄ , 1600 g CaCl ₂ , 240 g de MnSO ₄ , 240 g CuSO ₄ , 40 g CoSO ₄ , 240 g FeSO ₄ , 80 g NaMoO ₄ , 1040 g wood ash; 1 L milk, 1 kg brown sugar, 200 g beef liver, 200 g lime, 200 g natural phosphate.
Bio2	40 L fresh cattle manure; 60 L leaves, 1 kg brown sugar, 500 g natural phosphate, 1 L milk, 10 g yeast.
Bio3	100 L leaves 15 kg bone flour, 5 kg wood ash, 3 kg eggshells, 20 L milk, 20 kg brown sugar, 20 g yeast.

^aComposition for 100 L of water.

Table 3. Chemical properties of soil amended with biofertilizers.

	soil pH (1:2.5)	SOM (g kg ⁻¹)	P (mg kg ⁻¹)	K (cmolc kg ⁻¹)	Ca	Mg
at the beginning						
Bio1	6.3	5.1	3.0	0.9	1.81	5.1
Bio2	6.1	4.9	3.1	1.1	1.79	6.0
Bio3	6.3	5.2	3.0	1.0	1.70	5.9
NPK	6.0	5.3	3.9	0.9	1.84	5.3
Control	6.3	4.8	4.0	1.0	1.83	5.8
at the end						
Bio1	6.1	9.2	8.0	4.9	2.49	8.1
Bio2	6.1	8.0	7.5	2.4	2.13	7.0
Bio3	6.0	6.9	9.0	2.4	2.60	6.9
NPK	5.8	5.9	7.0	1.6	1.31	3.0
Control	5.9	4.0	4.5	1.1	1.42	3.9

SOM – soil organic matter

of the water holding capacity, with the goal of to equilibrate physiological activity of soil microbial biomass. The soil microbial biomass C (SMB-C) was determined according to Vance et al. (1987) with the 0.5 M K₂SO₄ extraction of the organic C from the fumigated and unfumigated soils. The coefficient of extraction of 0.38 was used to convert the difference in C between the fumigated and the unfumigated soil in microbial C. The soil respiration was monitored in aerobic incubation at 25 °C during seven days, through daily measurement of CO₂ evolution (Alef & Nannipieri, 1995). Fluorescein diacetate (FDA) hydrolysis was measured by spectrophotometry at 490 nm after incubation for 20 min at 30°C (Schnurer & Rosswall, 1982). The dehydrogenase (DHA) activity was determined using the method described by Casida et al. (1965), as based on the spectrophotometry determination of the triphenyl tetrazolium formazan released by 5 g of soil after 24 h incubation at 35°C.

The results are the means of determinations made of four replicates. Data were compared through analysis of variance (ANOVA). The means were compared by using least significant difference values calculated at the 5% level.

Results and Discussion

The biofertilizers presented different composition (Table 1) and content of nutrients (Table 2) and it affected significantly the soil chemical properties (Table 3). The chemical properties of soil showed an increase in soil organic matter and plant nutrients with the application of biofertilizers, mainly biofertilizer Bio1. The chemical properties of biofertilizers and soil also affected soil microbial properties. On the 10th, 40th and 60th days, the application of all biofertilizers increased soil microbial biomass C (SMB-C) and soil respiration as compared with both controls (Figure 1). In these evaluations, the highest and lowest values of SMB-C were found for the biofertilizers Bio1 (138, 188 and 191 mg C kg⁻¹, at 10th, 40th and 60th days) and Bio3 (105, 120 and 142 mg C kg⁻¹, at 10th, 40th and 60th days), respectively. Also, soil respiration showed the highest values with application of biofertilizer Bio1 (29.9, 29.1 and 40.1 mg CO₂ kg⁻¹, at 10th, 40th and 60th days), while the lowest values were found with Bio3 (23.1, 24.7 and 29.8 mg CO₂ kg⁻¹, at 10th, 40th and 60th days). At the end of evaluation, there were decreases in the SMB-C content and soil respiration for all soils treated with the biofertilizer.

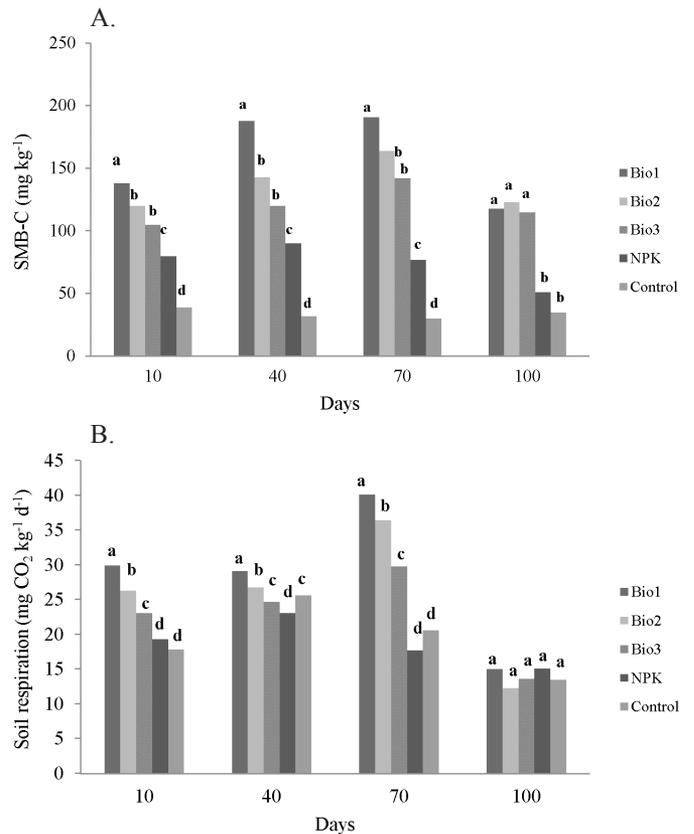


Figure 1. Soil microbial biomass C (SMB-C) (a) and soil respiration (b) after application of different biofertilizers

As expected, the results show that soil microbial biomass is stimulated by application of fresh organic matter with high C sources (Santos et al., 2012) and the higher SMB-C with application of biofertilizers compared to soil without addition of organic sources indicates a considerable incorporation of C into soil microbial biomass, which was also observed by Schweinsberg-Mickan & Müller (2009) who evaluated the effect of biofertilizers on soil microbial biomass. Also, soil respiration, measured by CO₂ release from soil, as an indicator of soil microbial activity (Alef, 1995) increased with addition of the biofertilizers.

On the other hand, the composition and quality of the biofertilizers led to differences in SMB-C and soil respiration found in Bio1 and Bio3. This can simply be explained by the presence of readily available C and nutrients contained in the Bio1 according to material used in its process of production (Table 1) and it reflected in the final chemical composition of this biofertilizer (Table 2). According to Sampaio et al. (2006), chemicals elements may contribute to the increase in soil microbial biomass due to the availability of nutrients for microbial cells. In addition, the higher availability of C sources in Bio1 stimulated the soil microbial biomass and activity. High availability of C sources favors soil microbial activity (Araujo et al., 2006). At the end of incubation, the decrease in soil respiration in soils amended with biofertilizers can be related to the microbial consumption of easily biodegradable C during the incubation period (Moreno et al., 1999).

Similar to soil microbial biomass and respiration, enzyme activities were highest with the application of biofertilizers.

However, on the 10th day, the values of enzymes activity did not differ between treatments (Figure 2). Afterward, on the 40th and 60th days, we found an increase in the values of FDA and DHA in the soil treated with the biofertilizer Bio1. In this case, the values of FDA hydrolysis and DHA activity were 11.2 and 13.7 mg FDA kg⁻¹ soil and 0.63 and 0.65 mg TTC kg⁻¹ soil, respectively. For the treatments with biofertilizers, the lowest values were found for biofertilizer Bio3. At the end of the period of evaluation, we observed decreased in FDA hydrolysis and dehydrogenase activity in all soils treated with biofertilizers.

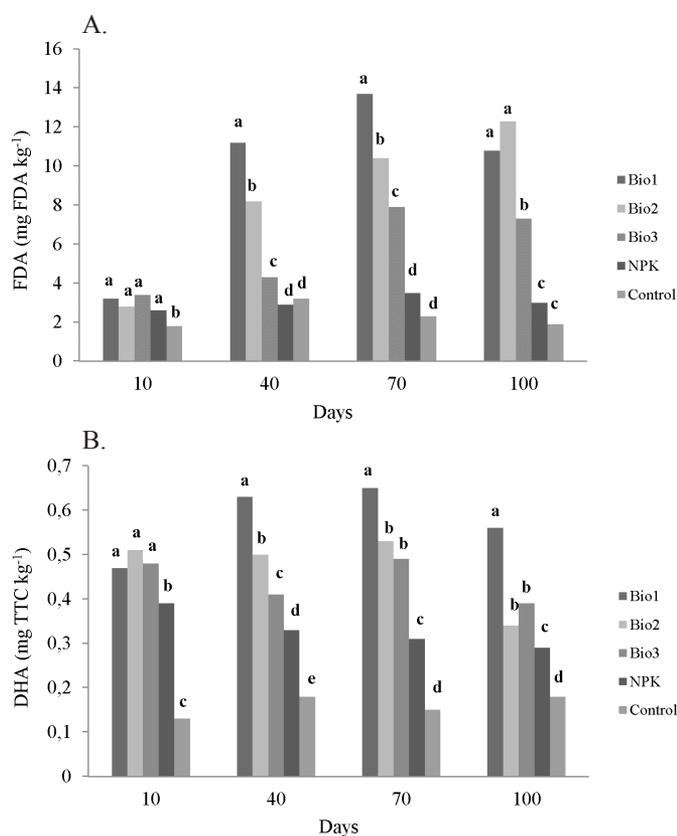


Figure 2. Soil FDA hydrolysis (a) and DHA activity (b) after application of different biofertilizers

The soil dehydrogenase activity reflects soil oxidative power and provides a way to quantify the total number of viable microbial cells (Gianfreda et al., 2005). FDA hydrolysis reflects the activity of several enzymes including lipases, esterases and proteases (Schnurer & Rosswall, 1982). Both parameters together are a good estimation of total soil microbial biological activity (Burns, 1978). These results indicate that the soils amended with biofertilizers presented more metabolically active microorganisms and, consequently, higher biological activity. Since that the high microbial activity of these enzymes are related to the decomposition of organic substrates, it indicates that in long-term there is a trend to increase soil fertility (Caravaca et al., 2004).

Conclusion

The application of biofertilizers increases the soil microbial biomass and activity. However, there are different responses of

soil microbial biomass to the different composition and quality of biofertilizers. In this study, the biofertilizer Bio1 promoted the best response of soil microbial biomass and activity.

Acknowledgment

The authors thank to CNPq and CAPES for the financial support to research and for master and research fellowship.

Literature Cited

- Alef, K.; Nannipieri, P. Methods in soil microbiology and biochemistry. New York: Academic Press, 1995. 576p.
- Alef, K. Estimation of soil respiration. In: Alef, K.; Nannipieri, P. Methods in soil microbiology and biochemistry. New York: Academic Press, 1995. p.464-470.
- Araujo, A.S.F.; Cesarz, S.; Leite, L.F.C.; Borges, C.D.; Tsai, S.M.; Eisenhauer, N. Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. *Soil Biology & Biochemistry*, v.66, p.175-181, 2013. <<http://dx.doi.org/10.1016/j.soilbio.2013.07.013>>.
- Araujo, A.S.F.; Monteiro, R.T.R. Microbial biomass and activity in a Brazilian soil amended with untreated and composted textile sludge. *Chemosphere*, v.64, n.6, p.1043-1046, 2006. <<http://dx.doi.org/10.1016/j.chemosphere.2006.01.040>>.
- Barros, L.E.O.; Liberalino Filho, J. Composto orgânico sólido e em suspensão na cultura do feijão-mungo verde (*Vigna radiata*). *Revista Verde*, v.3, p.114-122, 2008. <<http://www.gvaa.com.br/revista/index.php/RVADS/article/viewFile/68/68>>. 12 Mar. 2014.
- Burns R.G. Soil enzymes. New York: Academic Press, 1978. 370p.
- Burns, R.G.; Deforest, J.L.; Marxsen, J.; Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, M.D.; Weintraub, M.N.; Zoppini, A. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology & Biochemistry*, v.58, p.216-234, 2013. <<http://dx.doi.org/10.1016/j.soilbio.2012.11.009>>.
- Caravaca, F.; Barea, J.M.; Palenzuela, J.; Figueroa, D.; Alguail, M.N.; Roldán, A. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Applied Soil Ecology*, v.2, n.2, p.103-111, 2003. <[http://dx.doi.org/10.1016/S0929-1393\(02\)00136-1](http://dx.doi.org/10.1016/S0929-1393(02)00136-1)>.
- Casida, L.E.; Klein, A.; Santoro, T. Soil dehydrogenase activity. *Soil Science*, v.98, n.6, p.371-376, 1964. <<http://dx.doi.org/10.1097/00010694-196412000-00004>>.
- Chiconato, D.A.; Simoni, F.; Galbiatti, J.A.; Franco, C.F.; Caramelo, A.D. Resposta da alface à aplicação de biofertilizante sob dois níveis de irrigação. *Bioscience Journal*, v.29, n.2, p.392-399, 2013. <<http://www.seer.ufu.br/index.php/biosciencejournal/article/view/14077/12277>>. 12 Mar. 2014.
- Coimbra, K.G.; Peixoto, J.R.; Santin, M.R.; Nunes, M.S. Efeito de produtos alternativos no desempenho agrônomo de tomate rasteiro. *Bioscience Journal*, v.29, n.5, suplemento 1, p.1508-1513, 2013. <<http://www.seer.ufu.br/index.php/biosciencejournal/article/view/15206/13296>>. 05 Mar. 2014.

- El-Yazeid, A.A.; Abou-Aly, H.A.; Mady, M.A.; Moussa, S.A.M. Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. *Research Journal of Agricultural Biological Science*, v.3, n.4, p.274-286, 2007. <http://www.bu.edu.eg/portal/uploads/Agriculture/Botany/1156/publications/Hamed%20El-Sayed%20Abou%20Aly_PAPER_11.pdf>. 10 Mar. 2014.
- Empresa Brasileira de Pesquisa Agropecuária - Embrapa. Manual de análises químicas de solos, plantas e fertilizantes. Brasília, DF: Embrapa Informação Tecnológica, 2009. 627p.
- Gianfreda, L.; Rao, M.A.; Piotrowska, A.; Palumbo, G.; Colombo, C. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. *Science of Total Environment*, v.341, n.1-3, p.265-279, 2005. <<http://dx.doi.org/10.1016/j.scitotenv.2004.10.005>>.
- Jbhiye, R.P.; Sharma, R.R.; Tewari, R.N. Effects of biofertilizers on the growth and yield parameters of tomato. *Indian Journal of Horticulture*, v.60, n.4, p.368-371, 2003. <<http://www.indianjournals.com/ijor.aspx?target=ijor:ijh&volume=60&issue=4&article=014>>. 05 Mar. 2014.
- Mohammadi, K.; Sohrabi, Y. Bacterial biofertilizers for sustainable crop production: a review. *Journal of Agricultural Biological Science*, v.7, n.5, p.307-316, 2012. <http://www.arpnjournals.com/jabs/research_papers/rp_2012/jabs_0512_396.pdf>. 05 Mar. 2014.
- Moreno, J.L.; Hernandez, T.; Garcia, C. Effects of cadmium contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. *Biology & Fertility of Soils*, v.28, n.3, p.230-237, 1999. <<http://dx.doi.org/10.1007/s003740050487>>.
- Sampaio, D.B.; Araújo, A.S.F.; Santos, V.B. Avaliação de indicadores biológicos de qualidade do solo sob sistemas de cultivo convencional e orgânico de frutas. *Ciencia & Agrotecnologia*, v.32, n.2, p.353-359, 2008. <<http://dx.doi.org/10.1590/S1413-70542008000200001>>.
- Santos, V.B.; Araújo, A.S.F.; Leite, L.F.C.; Nunes, L.A.P.L.; Melo, W.J. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, v.170, p.227-231, 2012. <<http://dx.doi.org/10.1016/j.geoderma.2011.11.007>>.
- Schnurer, J.; Rosswall, T. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied & Environmental Microbiology*, v.43, n.6, p.1256-1261, 1982. <<http://aem.asm.org/content/43/6/1256.full.pdf+html>>. 12 Mar. 2014.
- Schweinsberg-Mickan, M.S.; Müller, T. Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth. *Journal of Plant Nutrition & Soil Science*, v.172, n.5, p.704-712, 2009. <<http://dx.doi.org/10.1002/jpln.200800021>>.
- Silva, D.I.B.; Nunes, L.A.P.L.; Araújo, A.S.F.; Oliveira, M.E.; Carneiro, R.F.V. Qualidade superficial de um solo sob sistemas de manejo utilizados para produção de forragens no estado Piauí. *Bioscience Journal*, v. 29, n.4, p.867-875, 2013. <<http://www.seer.ufu.br/index.php/biosciencejournal/article/viewFile/13520/12893>>. 05 Mar. 2014.
- Tedesco, M.J.; Gianello, C.; Bissani, C.A. Análises de solos, plantas e outros materiais. UFRGS: Porto Alegre, 1995.
- Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry*, v.19, n.6, p.703-707, 1987. <[http://dx.doi.org/10.1016/0038-0717\(87\)90052-6](http://dx.doi.org/10.1016/0038-0717(87)90052-6)>.
- Vessey, J.K. Plant growth promoting rhizobacteria as biofertilizers. *Plant & Soil*, v.255, n.2, p.571-586, 2003. <<http://dx.doi.org/10.1023/A:1026037216893>>.
- Yeomans, J.C.; Bremner J.M. A rapid and precise method for routine determination of organic carbon in soil. *Communication in Soil Science & Plant Analysis*, v.19, n.13, p.1467-1476, 1998. <<http://dx.doi.org/10.1080/00103628809368027>>.