

## Full Length Research Paper

## Short-term effect of different green manure on soil chemical and biological properties

Nilza Silva Carvalho, Antônio Bruno Bitencourt Oliveira, Maristella Moura Calaço Pessoa, Vicente Paulo Costa Neto, Ricardo Silva de Sousa, João Rodrigues da Cunha, Arthur Gonçalves Coutinho, Vilma Maria dos Santos and Ademir Sergio Ferreira de Araujo\*

Agricultural Science Center, Federal University of Piauí, Campus of Socopo, Teresina, Piauí, Brazil.

Received 6 May, 2015; Accepted 18 June, 2015

The use of different legume species, as green manure, may affect differently soil biological and chemical properties. The aim of this study was to evaluate the effect of four legumes species used as green manure on soil biological and chemical properties in short-term. We evaluated the following legume species: *Crotalaria*, *Cajanus*, *Mucuna* and *Canavalia*. The study was arranged in a completely randomised design with four replicates. The plants were incorporated into the soil (0-20 cm) by harrow and the chemical and biological properties were evaluated 30 and 60 days from the incorporation. Soil chemical and biological properties showed different trends according to legume species used. Soil P and K contents were highest in plot with *Crotalaria*, while soil Ca content was highest in plot with *Mucuna*. Soil microbial biomass was higher in plot with *Mucuna* as compared with others green manure species. Fluorescein diacetate hydrolysis was higher in plots with *Mucuna* and *Canavalia* than the others plots. Our results supported the hypothesis that different types of legume used as green manure affect differently the biological and chemical properties of soil. In this case, *Mucuna* was more effective to improve soil biological properties, while *Crotalaria* seems to be more efficient in the improvement of chemical properties.

**Key words:** Organic fertilization, alternative agriculture, legumes.

### INTRODUCTION

The use of legumes species as green manure is an important practice for sustainable agriculture as the legumes may fix N and have deep and extensive root system, allowing greater extraction and recycling of plant nutrients. Some studies have shown the great potential of green manure for supplying nutrients to crops (Perin et al., 2004; Adediran et al., 2004) and the improvement of soil properties (Adediran et al., 2004; Ziblim et al., 2013).

However, several legume species may be used as green manure and each one presents different properties which may affect differently the soil properties. Also, the influence of organic residues on soil properties depends upon the amount, type and size of the added organic materials (Zhang et al., 2015). Among the legume species used for green manure in tropical regions, the *Canavalia* is one of the most favorable for agricultural use

\*Corresponding author. E-mail address: asfaruaj@yahoo.com.br. Tel: +55-86-3215-5740. Fax: +55-86-3215-5743.

**Table 1.** Chemical properties of legume species.

Parameter	<i>Crotalaria</i>	<i>Guandu</i>	<i>Mucuna</i>	<i>Canavalia</i>
Dry mass (t ha <sup>-1</sup> )	9.0	8.5	13.4	8.1
C/N ratio	22	19	29	24
N (g kg <sup>-1</sup> )	20.1	16.3	21.2	18.7
P (g kg <sup>-1</sup> )	5.4	3.0	1.9	3.5
K (g kg <sup>-1</sup> )	25.7	13.4	13.0	12.4
Ca (g kg <sup>-1</sup> )	4.2	3.0	12.6	3.9
Mg (g kg <sup>-1</sup> )	2.0	1.3	2.8	3.0

by their morphological and physiological characteristics (Heinrichs et al., 2002), while *Crotalaria* is very efficient as producer of plant dry mass (Ziblim et al., 2013). *Mucuna* is other legume specie which has a potential for soil restoration, improving the chemical and physical properties of the soil (Alvarenga et al., 1995). Finally, *Cajanus* presents deep root system with high potential in water absorption and recycling of nutrients from deep layers (Alvarenga et al., 1995).

Although it is widely known that green manure and others crop residues improve the soil chemical and biological properties (Biederbeck et al., 2005; Liu et al., 2006; Shah et al., 2010; Ye et al., 2014; Adediran et al., 2004; Ziblim et al., 2013), there is the need to compare the different legume species in the improvement of soil biological and chemical properties in the short-term as usually these soil properties may respond quickly to soil management according to chemical characteristics of organic residue.

As an important biological property, soil microbial biomass is used as an ecological attribute to evaluate changes in soil properties by soil use and management (Lopes et al., 2010; Santos et al., 2012). In addition, soil microbial biomass releases enzymes which play important functions in soil processes, such as the decomposition of organic matter (Silva et al., 2012). Thus, soil enzyme activity may be used as an indicator of soil quality due to its control on microbial growth (Burns et al., 2013). For chemical properties, Smith and Doran (1996) suggested that soil total organic carbon (TOC), available P, exchangeable K and soil pH are important measurements of soil quality because they provide indicators of soil nutrient supplying capacity.

We hypothesized that each legume species presents different chemical composition and therefore affect differently soil biological and chemical properties in short-term. Thus, the aim of this study was to evaluate the effect of four legumes species used as green manure on soil biological and chemical properties in short-term.

## MATERIALS AND METHODS

The experiment was located at the Experimental Area from Agricultural Science Centre, Teresina, Piauí, Brazil. The regional

climate is dry tropical (Köppen) and is characterised by two distinct seasons: Rainy summer and dry winter, with annual average temperatures of 30°C and rainfall of 1200 mm. The rainy season extends from January to April, when 90% of the total annual rainfall occurs. The soil is classified as a Fluvisol with the following composition at 0 to 20 cm depth: 10% clay, 28% silt, and 62% sand.

The following legume species were evaluated: *Crotalaria*, *Cajanus*, *Mucuna* and *Canavalia*. We included a treatment without green manure as a reference (control). The experimental area was arranged in a completely randomised design with four replicates. Plots measured 10 m<sup>2</sup> each, with 6 m<sup>2</sup> of usable area for soil sampling and rows are spaced 0.4 m apart. *Crotalaria* and *Cajanus* were grown at a density of 10 plant m<sup>-1</sup>, while *Mucuna* and *Canavalia* were grown at a density of 7 plant m<sup>-1</sup>. After 60 days from the sowing (flowering), all plants inside the plots were harvested and incorporated into the soil (0-20 cm) by harrow. Chemical characteristics of the legume species are shown in Table 1 (Cavalcanti et al., 2012). After 30 and 60 days from the incorporation, five soil samples were collected (0-20 cm) in each plot (from usable area) using a spade and pooled to form a composite sample per plot. Each soil sample was sieved (< 2 mm) and stored prior to analysis. Soil pH was estimated in water (1:2.5 v:v) and measured using a pH meter (Tedesco et al., 1995). Available P and exchangeable K, Ca, and Mg were evaluated according with Tedesco et al. (1995). Soil organic C was determined by wet combustion using a mixture of 5 mL of 0.167 mol L<sup>-1</sup> potassium dichromate and 7.5 mL of concentrated sulphuric acid under heating (170°C for 30 min) (Yeomans and Bremner 1988).

Soil microbial biomass C (MBC) was determined according to the methods developed by Vance et al. (1987) with 0.5 M K<sub>2</sub>SO<sub>4</sub> extraction of the organic C contents from fumigated and unfumigated soils. The soil respiration was monitored through daily measurement of CO<sub>2</sub> evolution under aerobic incubation at 25°C for 7 days (Alef and Nannipieri, 1995). Fluorescein diacetate (FDA) hydrolysis was measured by spectrophotometry at 490 nm after incubation for 20 min at 30°C, according to the method described by Schnürer and Rosswall (1982).

The data were submitted to the analysis of variance (ANOVA) and the means were compared by the Student's test (5% level). All the statistical analyses were performed with the SPSS (version 15.0) software package. The data from the variables were analysed using multivariate ordination non-metric multidimensional scaling (NMS) with Sorensen distances. Ordination was performed using the PC-ORD v. 6.0 program.

## RESULTS

The incorporation of green manure changed the soil

**Table 2.** Chemical properties of soil after 60 days of incorporation of green manures.

Plots	pH	P*	K**	Ca**	Mg**
		mg kg <sup>-1</sup>		cmolc kg <sup>-1</sup>	
<i>Crotalaria</i>	7.3 <sup>a</sup>	62.4 <sup>a</sup>	16.4 <sup>a</sup>	2.2 <sup>b</sup>	0.7 <sup>a</sup>
<i>Guandu</i>	7.2 <sup>a</sup>	44.9 <sup>b</sup>	14.8 <sup>b</sup>	1.7 <sup>b</sup>	0.7 <sup>a</sup>
<i>Mucuna</i>	6.9 <sup>a</sup>	40.4 <sup>b</sup>	15.0 <sup>b</sup>	4.2 <sup>a</sup>	0.6 <sup>a</sup>
<i>Canavalia</i>	7.1 <sup>a</sup>	48.8 <sup>b</sup>	14.3 <sup>b</sup>	2.9 <sup>b</sup>	0.4 <sup>b</sup>
Control***	6.8 <sup>a</sup>	25.1 <sup>c</sup>	9.4 <sup>c</sup>	1.3 <sup>c</sup>	0.2 <sup>c</sup>

\* Available P; \*\* Exchangeable K, Ca, and Mg; \*\*\* Control – plot without green manure

**Table 3.** Soil organic C (SOC, g kg<sup>-1</sup>), microbial biomass (MBC, mg C kg<sup>-1</sup>), microbial quotient (qmic, %), basal respiration (BR, mg CO<sub>2</sub> kg<sup>-1</sup>), and diacetate fluorescein hydrolysis (FDA, mg FDA kg<sup>-1</sup>) in plots with different green manure.

Plots	SOC		MBC		qmic		BR		FDA	
	30	60	30	60	30	60	30	60	30	60
<i>Crotalaria</i>	6.6 <sup>a</sup>	5.9 <sup>a</sup>	80.3 <sup>b</sup>	98.5 <sup>b</sup>	1.2 <sup>b</sup>	1.6 <sup>a</sup>	5.06 <sup>a</sup>	3.56 <sup>a</sup>	74.1 <sup>b</sup>	81.4 <sup>b</sup>
<i>Guandu</i>	6.3 <sup>a</sup>	5.8 <sup>a</sup>	83.6 <sup>b</sup>	107.6 <sup>b</sup>	1.3 <sup>b</sup>	1.8 <sup>a</sup>	4.97 <sup>a</sup>	3.55 <sup>a</sup>	78.7 <sup>b</sup>	96.0 <sup>b</sup>
<i>Mucuna</i>	6.2 <sup>a</sup>	6.7 <sup>a</sup>	132.5 <sup>a</sup>	138.5 <sup>a</sup>	1.6 <sup>a</sup>	1.5 <sup>a</sup>	5.19 <sup>a</sup>	3.71 <sup>a</sup>	154.1 <sup>a</sup>	142.9 <sup>a</sup>
<i>Canavalia</i>	6.5 <sup>a</sup>	6.8 <sup>a</sup>	78.2 <sup>b</sup>	113.2 <sup>b</sup>	1.2 <sup>b</sup>	1.6 <sup>a</sup>	5.23 <sup>a</sup>	4.69 <sup>a</sup>	175.2 <sup>a</sup>	158.1 <sup>a</sup>
Control*	6.1 <sup>a</sup>	6.3 <sup>a</sup>	46.3 <sup>c</sup>	39.2 <sup>c</sup>	0.6 <sup>c</sup>	0.5 <sup>c</sup>	4.81 <sup>a</sup>	4.06 <sup>a</sup>	52.1 <sup>c</sup>	47.3 <sup>c</sup>

\*Control, plot without green manure.

chemical properties as compared with soil without green manure (Table 2), where the incorporation of green manure increased the content of available P and exchangeable K, Ca and Mg. Comparing the plots with green manure, soil chemical properties differed according to the legume species used (Table 2). Soil pH did not differ between evaluated plots. However, available P and exchangeable K contents were highest in plot with *Crotalaria*, while soil exchangeable Ca content was highest in plot with *Mucuna*.

The incorporation of green manure, in short-term, did not promote difference in soil organic C content at 30 and 60 days after green manure application. Soil biological properties changed with the incorporation of green manure (Table 3). The incorporation of green manure promoted an increase in MBC as compared with soil without green manure. However, soil MBC showed different behavior according to the green manure incorporated. Thus, plots with *Mucuna* showed highest values of MBC at 30 and 60 days as compared with others green manure species. There was an increase in soil MBC from the 30<sup>th</sup> to 60<sup>th</sup> days of evaluation for all plots with green manure. At 30<sup>th</sup> day, the microbial quotient (qmic) was higher in plot with *Mucuna*; while at the 60<sup>th</sup> day there was not difference between plots.

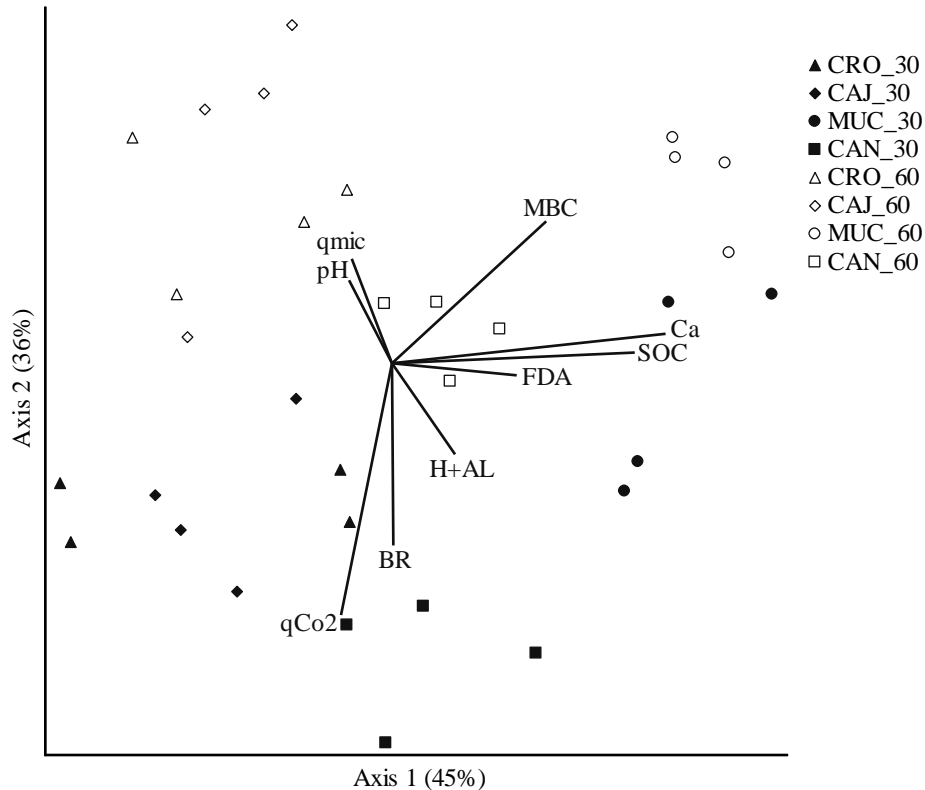
Soil microbial activity, as measured by basal respiration and FDA hydrolysis, increased with the use of green manure as compared with the plot without green manure (Table 3). However, the soil basal respiration did not

differ between plots with green manure. On the other hands, FDA hydrolysis was higher in plots with *Mucuna* and *Canavalia* than the others plots. There were not differences in values of FDA hydrolysis between the 30<sup>th</sup> and 60<sup>th</sup> days of evaluation for all evaluated plots.

NMS analysis explained 81% of variation and clustered two main groups according to biological and chemical properties (Figure 1). The first group consisted of plot with *Mucuna* and was linked with soil microbial biomass C and FDA hydrolysis; while the other group consisted of plots with *Guandu*, *Crotalaria* and *Canavalia* and was grouped with soil basal respiration and microbial quotient.

## DISCUSSION

The incorporation of green manure, in short-term, increased the content of some chemical elements in the soil, suggesting that this practice may improve the soil fertility shortly. Previous studies had shown the positive effect of green manure on soil fertility in short-term (Astier et al., 2006; Partey et al., 2014). The legume species promoted different responses of soil chemical properties and it occurred due to different composition of each green manure. The higher values reported for soil P and K content in plot with *Crotalaria* are due to decomposition of the green manure, which presents high content of P and K in its composition (Table 1) and contributes for the releasing of these elements to the soil. Similar results



**Figure 1.** NMS of chemical and biological properties of soil after the incorporation of different green manures.

were found by Ziblim et al. (2013) comparing the potentials of *Mucuna* and *Crotalaria* as green manure on soil fertility, where they observed that *Crotalaria* added a higher amount of available P and K as compared to *Mucuna*. It is important for the improvement of soil fertility and may provide essential nutrient for crops. On the other hands, *Mucuna* presented high Ca content in its composition and it contributes for the increase in soil Ca in soil with this legume specie. These findings are in agreement with Adediran et al. (2004) which evaluated the effect of *Mucuna* intercropped with maize (*Zea mays* L.) on soil fertility and observed, after two months of *Mucuna* application, the increasing in the content of exchangeable Ca by 81% when compared with the chemically fertilized soil.

Our results showed that soil organic C did not change, in short-term, with the addition of green manure indicating that soil organic matter is not sensitive to short-term changes of soil quality with different soil or crop management practices (Liu et al., 2013). Usually, soil organic C increases in medium- to long-term as reported in previous studies (Dou et al., 2006; Huang et al., 2010).

On contrary, soil microbial biomass is a sensitive indicator of soil quality and responds quickly to changes in soil management. Several studies reported that soil microbial biomass improved significantly by using of

legumes as green manure (Biederbeck et al., 2005; Liu et al., 2006; Shah et al., 2010). The results showed that soil microbial biomass and showed different values according to legume specie as also reported by Shah et al. (2010) who evaluated six types of legumes as green manure and observed that soil microbial biomass varied with the type of legume, where the highest microbial biomass was found in soil with sesbania and lowest in plot with guar. In our study, the higher soil MBC found in plots with *Mucuna* occurred due to its high input of dry mass with low C/N ratio which increases the availability of C to soil microorganisms (Franchini et al., 2007). It may suggest that this legume specie presents high potential to improve soil biological properties as reported by Blanchart et al. (2006) who evaluated the use of *Mucuna* as cover crop and found a positive modification in diversity and composition of soil biota.

On the other hands, the increase in soil microbial biomass from 30<sup>th</sup> to 60<sup>th</sup> day suggests that the all green manure maintained C source to soil microbial biomass. It may have occurred since the legumes are applied on soil surface and releases slowly the C for soil microbial consumption. Therefore, there is an increasing in soil microbial biomass over-time. The highest microbial quotient observed in plot with *Mucuna* is due the higher soil microbial biomass found in this plot. As the microbial

quotient is an indicator of availability of organic C for soil microorganism (Anderson and Domsch, 1989), this result means that in plot with *Mucuna* the organic C is more available for soil microbial biomass (Steiner et al., 2008).

Soil management practices which modify the soil microbial biomass also affect the enzyme activities (Dick et al., 1996). The FDA hydrolysis is directly proportional to the microbial growth (Swisher and Carroll, 1980) and is involved in the transformation of soil organic matter (Sicardi et al., 2004). Also, FDA hydrolysis is a good indicator of soil microbial activity and reflects the activity of several enzymes, including lipases, esterases and proteases, and its activity increases with SMB (Schnurer and Rosswall, 1982). Our results showed that microbial metabolism was positively influenced by *Mucuna* and *Canavalia*, suggesting these legume species may increase the soil microbial activity.

The NMS analyses of the soil biological and chemical properties revealed distinct trends for the legume species used as green manure. The plot with *Mucuna* clustered, at 30<sup>th</sup> and 60<sup>th</sup> days, and was characterized by higher microbial biomass C and microbial activity. This may suggest that this legume species has a potential to improve soil biological properties. On the other hand, *Crotalaria*, *Guandu* and *Canavalia* clustered together and were characterized by basal respiration and microbial quotient, which can be indicative of higher decomposition and availability of C and others nutrients.

## Conclusion

Our results supported the hypothesis that different types of legume used as green manure affect differently the biological and chemical properties of soil. It occurs mainly due the different chemical composition of each legume species used. In this case, *Mucuna* was more effective to improve soil biological properties, while *Crotalaria* seems to be more efficient in the improvement of chemical properties.

## Conflict of Interests

The authors have not declared any conflict of interests.

## REFERENCES

- Adediran JA, Akande MO, Oluwatoyinbo FI (2004). Effect of mucuna intercropped with maize on soil fertility and yield of maize. *Ghana J. Agric. Sci.* 37:15-22.
- Anderson JPE, Domsch KH (1989). Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biol. Biochem.* 21:471-479.
- Alef K, Nannipieri P (1995). *Methods in Soil Microbiology and Biochemistry*, Academic Press, New York pp. 464-470.
- Alvarenga RC, Costa LM, Moura Filho W, Regazzi AJ (1995). Características de alguns adubos verdes de interesse para a conservação e recuperação de solos. *Pesq. Agropec. Bras.* 30:175-185.
- Astier M, Maass JM, Etchevers-Barra JD, Pena JJ, Gonzalez FL (2006). Short-term green manure and tillage management effects on maize yield and soil quality in an Andisol. *Soil Till. Res.* 88:153-159
- Biederbeck VO, Zentner RP, Campbell CA (2005). Soil microbial populations and activities as influenced by legume green fallow in a semiarid climate. *Soil Biol. Biochem.* 37:1775-1784.
- Blanchart E, Villenave C, Viallatoux D, Brathes B, Girardin C, Azontonde A, Feller C (2006). Long-term effect of a legume cover crop (*Mucuna pluriens*) on the community of soil macrofauna and nematofauna in maize cultivation in Southern Benin. *Euro. J. Soil Biol.* 42:136-144.
- Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stronberger ME, Wallenstein MD, Weintraubg MN, Zoppini A (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.* 58:216–234.
- Cavalcante VS, Santos VR, Santos Neto AL, Santos MAL, Santos CG, Costa LC (2012). Biomassa e extração de nutrientes por plantas de cobertura. *Rev. Bras. Eng. Agríc. Amb.* 16:521-528.
- Dick RP, Breakwell DP, Turco RF (1996). Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran JW, Jones AJ (eds) *Methods of assessing soil quality*. Soil Science Society of America, Special Publication 49, Madison, WI. pp. 247-271.
- Dou F, Hons FM (2006). Tillage and nitrogen effects on soil organic matter fractions in wheat-based systems. *Soil Sci. Soc. Am. J.* 70:1896-1905.
- Franchini JC, Crispino CC, Souza RA, Torres E, Hungria M (2007). Microbiological parameters as indicators of soil quality under various tillage and crop-rotation systems in southern Brazil. *Soil Till. Res.* 92:18-29.
- Heinrichs R, Vitti GC, Moreira A, Fancelli AL (2002). Produção e estado nutricional do milho em cultivo consorciado intercalar com adubos verdes. *Rev. Bras. Ci. Solo* 26:225-230.
- Huang S, Sun Y, Rui W, Liu W, Zhang W (2010). Long-term effect of no-tillage on soil organic carbon fractions in a continuous maize cropping system of Northeast China. *Pedosphere* 20:285-292.
- Liu E, Yan C, Mei X, Zhang Y, Fan T (2013). Long-term effect of manure and fertilizer on soil organic carbon pools in dryland farming in northwest china. *PLoS ONE* 8:e56536.
- Liu GS, Luo ZB, Wang Y, Li HL, Wang GF, Ma JM (2006). Effect of green manure application on soil properties and soil microbial biomass in tobacco field. *J. Soil Water Conserv.* 20:95-98.
- Lopes MM, Salviano AAC, Araujo ASF, Nunes LAPL, Oliveira ME (2010). Changes in soil microbial biomass and activity in different Brazilian pastures. *Spanish J. Agric. Res.* 8:1253-1259.
- Partey ST, Preziosi RF, Robson GD (2014). Short-term interactive effects of biochar, green manure, and inorganic fertilizer on soil properties and agronomic characteristics of maize. *Agric. Res.* 3:128-136
- Perin A, Santos RHS, Urquiaga S, Guerra JGM, Cecon PR (2004). Efeito residual da adubação verde no rendimento de brócolo (*Brassica oleracea* L. var. Itálica) cultivado em sucessão ao milho (*Zea mays* L.). *Ci. Rural* 34:1739-1745.
- Santos VB, Araujo ASF, Leite LFC, Nunes LAL, Melo WJ (2012). Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma* 170:227-231.
- Schnurer J, Rosswall T (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* 43:1256-1261.
- Shah Z, Ahmad SR, Rahman HU (2010). Soil microbial biomass and activities as influenced by green manure legumes and N fertilizer in rice-wheat system. *Pak. J. Bot.* 42:2589-2598.
- Sicardi M, García-Prechac F, Frioni L (2004). Soil microbial indicators sensitive to land use conversion from pastures to commercial *Eucalyptus grandis* (Hill ex Maiden) plantations in Uruguay. *Appl. Soil Ecol.* 27:125-133.
- Silva DKA, Freitas NO, Sousa RG, Silva FSB, Araujo ASF, Maia LC (2012). Soil microbial biomass and activity under natural and regenerated forests and conventional sugarcane plantations in Brazil. *Geoderma* 189:257-261.
- Smith JL, Doran JW (1996). Measurement and use of pH and electrical conductivity for soil quality analysis. Doran JW, Jones AJ (Eds). *Methods for Assessing Soil Quality*. Madison: SSSA 49:41-50.

- Steiner C, Das KC, Garcia M, Forster B, Zech W (2008). Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia* 51:359-366.
- Tedesco MJ, Gianello C, Bissani CA (1995). Análises de solos, plantas e outros materiais. UFRGS: Porto Alegre P 230.
- Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19:703-707.
- Yeomans JC, Bremner JM (1998) A rapid and precise method for routine determination of organic carbon in soil. *Comm. Soil Sci. Pl. Anal.* 19:1467-1476.
- Zhang Q, Zhou W, Liang G (2015) Effects of different organic manures on the biochemical and microbial characteristics of albic paddy soil in a short-term experiment. *PLoS ONE* 10:e0124096.
- Ziblim IA, Paul GS, Timothy KA (2013) Assessing soil amendment potentials of *Mucuna pruriens* and *Crotalaria juncea* when used as fallow crops. *J. Soil Sci. Environ. Manage.* 4:28-34.