DOI: 10.5433/1679-0359.2018v39n3p999

Changes in the stocks of C and N in organic matter fractions in soil cropped with coffee and fertilized with sunn hemp and ammonium sulfate

Alterações dos estoques de C e N nas frações da matéria orgânica de solo cultivado com cafeeiro e adubado com crotalária e sulfato de amônio

Wander Douglas Pereira[1*]; Fábio Lúcio Martins Neto[2]; Ricardo Henrique Silva Santos[3]; Teógenes Senna de Oliveira[4]; Segundo Sacramento Urquiaga Caballero[5]

Abstract

Despite the potential to provide N to crops, the rapid incorporation of green manure nutrients into stable fractions of organic matter in the soil (SOM) may reduce the efficiency of green manuring. Thus, the objective of this work was to characterize the changes of C and N stocks in fractions of SOM cultivated with coffee (*Coffea arabica* L.) and fertilized with sunn hemp (*Crotalaria juncea*) and ammonium sulfate. To study the changes in organic C (OC) and total N (TN) in soil and fractions of SOM over time, soil samples were collected in the 0–5 and 5–10 cm layers, with the initial sampling done prior to the application of sunn hemp residues and ammonium sulfate. Five samples were collected every 2 months after the application of the legume and ammonium sulfate. The soil samples were submitted to densimetric and granulometric fractionation, obtaining the free light organic matter (F-LOM), particulate organic matter (POM), and organic matter associated with minerals (MAM). OC and TN stocks were then determined in soil and the SOM fractions. The changes in the stocks of OC (ΔStcC) in the soil in relation to time zero were positive in the evaluations carried out in the two layers. The fractions of SOM showed positive ΔStcC at almost all of the evaluated times. The N supplied to the soil in the form of mineral and organic fertilizer promoted an increase of 0.24 Mg ha^{-1} of N in the 0–5 cm layer until after 60 days. Of this total, 0.03 Mg ha^{-1} was associated with F-LOM, 0.07 Mg ha^{-1} with POM, and the remainder was associated with MAM. Nearly 60% of the N that was supplied to the soil was drawn to the stable fractions of the SOM, indicating a rapid stabilization of this nutrient in the most recalcitrant organic compartments. Despite that, the variations in N stocks of MAM became smaller over time, and eventually became negative, in relation to time zero. This indicates the mineralization of N of this compartment. In the 5–10 cm layer, no effect of time was observed in the soil TN, N-POM, or N-MAM stocks. Additionally, under the conditions of this experiment, the majority of the N supplied to the soil was rapidly incorporated into the most stable fraction of SOM, and this might can reduced the efficiency of the green manuring.

Key words: Green manure. Organic compartments. *Crotalaria juncea*. Densimetric fractionation. Granulometric fractionation.

[1] Engº Agrº, M.e, Departamento de Fitotecnia, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: wanderdp@yahoo.com.br

[2] Discente, Curso de Doutorado, Programa de Pós-Graduação em Fitotecnia, UFV, Viçosa, MG, Brasil. E-mail: fabiopiata@yahoo.com.br

[3] Prof. Dr., Departamento de Fitotecnia, UFV, Viçosa, MG, Brasil. E-mail: rsantos@ufv.br

[4] Prof. Dr., Departamento de Solos, UFV, Viçosa, MG, Brasil. E-mail: teo@ufv.br

[5] Engº Agrº, Dr. Pesquisador, Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Agrobiologia, Seropédica, RJ, Brasil. E-mail: segundo.urquiaga@embrapa.br

[*] Author for correspondence

Received: Sept. 05, 2017 - Approved: Feb. 28, 2018

Resumo

Apesar do potencial para fornecer N aos cultivos, a rápida incorporação dos nutrientes do adubo verde em frações estáveis da matéria orgânica do solo (MOS) pode reduzir a eficiência da adubação verde. Portanto, objetivou-se com este trabalho caracterizar as alterações temporais dos estoques de C e N em frações da matéria orgânica de solo cultivado com cafeeiro (*Coffea arabica* L.) e adubado com crotalária (*Crotalaria juncea*) e sulfato de amônio. Para estudar o comportamento do C orgânico (CO) e N total (NT) no solo e nas frações da MOS ao longo do tempo, foram coletadas amostras de solo nas camadas de 0–5 e 5–10 cm, sendo a primeira amostragem antes da aplicação dos resíduos de crotalária e sulfato de amônio. Outras cinco amostras foram coletadas a cada dois meses após a aplicação da leguminosa e sulfato de amônio. As amostras de solo foram submetidas ao fracionamento densimétrico e granulométrico, obtendo-se as frações matéria orgânica leve livre (MOLL), matéria orgânica particulada (MOP) e matéria orgânica associada aos minerais (MAM). Foram determinados os estoques de CO e NT no solo e nas frações da MOS. As variações nos estoques de C (ΔEstC) orgânico no solo, em relação ao tempo zero, foram positivas nas avaliações realizadas nas duas camadas. As frações da MOS apresentaram ΔEstC positivas em quase todos os tempos de avaliação. O N aportado ao solo na forma de fertilizante mineral e orgânico promoveu incremento de 0,24 Mg ha^{-1} de N na camada de 0–5 cm até os 60 dias. Desse total, 0,03 Mg ha^{-1} estavam associados à MOLL, 0,07 Mg ha^{-1} à MOP e o restante estava associado à MAM. Quase 60% do N que foi aportado ao solo teve como destino frações estáveis da MOS, indicando rápida estabilização desse nutriente nos compartimentos orgânicos mais recalcitrantes. Apesar disso, com o passar do tempo as variações nos estoques de N da MAM tornaram-se menores, e posteriormente negativas, em relação ao tempo zero, indicando a mineralização do N desse compartimento. Na camada de 5–10 cm não houve efeito do tempo nos estoques de NT do solo, N-MOP ou N-MAM. A adubação com *C. juncea* proporciona incrementos nos estoques de C de frações lábeis e recalcitrantes da MOS. Também, nas condições desse experimento, a maior parte do N aportado ao solo é rapidamente incorporado às frações mais estáveis da MOS, o que pode reduzir a eficiência da adubação verde.

Palavras-chave: Adubação verde. Compartimentos orgânicos. *Crotalaria juncea*. Fracionamento densimétrico. Fracionamento granulométrico.

Introduction

N is typically the most essential nutrient in agriculture. In addition to the high demand for fossil fuels and energy for their acquisition, the use of synthetic N fertilizers increases the costs of production (EFMA, 2000), which compromises their sustainable use. Green manuring is an agricultural practice that may benefit the agroecosystem by improving soil fertility through the contribution of N via biological fixation and the increase of soil organic matter (SOM) (RICCI et al., 2005; DELARMELINDA et al., 2010; PERIN et al., 2010; PARTELLI et al., 2011; ARAÚJO et al., 2014). Therefore, green manuring is a strategic tool in soil fertility management, especially in production systems that excel in sustainability.

Despite the potential of N input into the system, studies frequently demonstrate the low efficiency of N recovery of legumes in crops. The values were less than 25% (SEO et al., 2006; AMBROSANO et al., 2011; ARAÚJO et al., 2011; DINIZ et al., 2015), whereas in the soil and the remaining material, higher amounts of N derived from the legume were recovered (ARAÚJO et al., 2005, 2011; SEO et al., 2006; LANGE et al., 2009).

Considering that the majority of N in the soil is in its organic form (MYROLD, 2005); therefore, it is fundamental to understand the alterations in SOM after fertilization with legumes. It is also important to recognize the changes for N and C in the soil since a relationship exists between SOM, C, and N. Increases in organic C (OC) stocks are directly related to increases in soil N, which is fundamental for C stabilization by means of formation of humified substances, which are richer in N than other less recalcitrant organic compartments (LOVATO et al., 2004; RIBEIRO et al., 2011; GAUDER et al., 2016).

The ease in which SOM compartments are decomposed will define how their N can be used over time. As a result, the fractionation of SOM becomes of central importance. One way to examine SOM is through its physical fractionation. This procedure allows the separation of organic fractions according to their physical protection in the soil aggregates and their chemical stability with the mineral components; in turn, this information can be used to relate organic fractions to their function in the soil (CHRISTENSEN, 2001; SOHI et al., 2001).

The light free organic matter (F-LOM) is the most sensitive compartment to indicate alterations caused by agricultural practice, compared to the total organic C (TOC) and that associated with heavy fractions (organic-mineral associations) (MARIN et al., 2006; SOUZA et al., 2006). The organic-mineral associations (silt and clay) tend to vary little with management practices (SOUZA et al., 2006).

The nutrient mineralization of legume residues is rapid in tropical climates (PERIN et al., 2010), suggesting that it is possible that C and N - added via green manure and mineralized - have the most stable SOM compartments as their rapid final destination.

In temperate climates, studies showed that C and N added to the soil via plant residue were rapidly incorporated into the organo-mineral fractions of silt and clay (KÖLBL et al., 2006; BIMÜLLER et al., 2013; POIRIER et al., 2014). The rapid incorporation of N to more stable organic fractions may reduce the efficiency of green manure in crop nutrition, despite enhancing soil properties. In tropical climates, such a process can occur even faster, modifying the possibility of crop nutrition in the short term.

The objective of this work was to characterize the temporal changes of C and N in fractions of SOM cultivated with coffee (*Coffea arabica* L.) and fertilized with *C. juncea* and ammonium sulfate.

Material and Methods

The experiment was conducted in a protected area of the Department of Plant Science (Fitotecnia) of the Universidade Federal de Viçosa (UFV), Viçosa, State of Minas Gerais, Brazil, from January 7th to November 20th, 2013. The geographic coordinates are 20° 45′ 14″ S and 42° 52′ 53″ W at 650 m above sea level.

The experimental unit consisted of a 150 dm^3 box with 0.59 m^2 of upper area, containing a coffee plant (*Coffea arabica* L.) 'Catuaí 44' at 2 months of age at transplanting. The experiment consisted of five replicates in a completely randomized design, with treatments and assessments over time.

The soil material used in this study had a clay-sandy texture from the cropped area close to the experiment. Field collection and the filling of the box considered the maintenance of the 0–20 cm and 20–40 cm layers collected in the field. Corrections were made according to the Comissão de Fertilidade do Solo do Estado de Minas Gerais (Soil Fertility Commission of the State of Minas Gerais) (CFSEMG) (GUIMARÃES et al., 1999), according to the chemical and physical characteristics of the collected soil material (Table 1).

The boxes were irrigated to maintain soil moisture between 70 and 80% of its field capacity, measured by the ECH_2O dielectric sensor, calibrated according to Miranda et al. (2007). The bottoms of the boxes were filled with expanded clay to allow the drainage of excess water and the subsequent return of percolated water to the boxes.

Before transplanting the coffee plants, 65 g of P_2O_5 was applied to the soil in each box (50% as simple superphosphate and 50% as reactive rock phosphate). At planting, 6 g of N per plant was applied (60% of the N via ammonium sulfate and 40% as the dry matter of aboveground part of sunn hemp (*C. juncea*)). A recovery efficiency of 50% of mineral N was considered, implied by the application of 7.2 g of N. Regarding sunn hemp biomass and considering values reported in the literature, the recovery efficiency adopted was 25% (SEO et al., 2006; AMBROSANO et al., 2011; ARAÚJO et al., 2011), resulting in a dose of 9.6 g of N. The applied quantity of residues corresponded to 280 g of crushed residues from the aboveground part of sunn hemp, corresponding to a dose of 4.75 Mg ha^{-1}. The legume exhibited a concentration of 3.4% N in the dry matter, and it had been produced primarily for experimental use.

Table 1. Chemical and physical characteristics of soil matter used in different depths.

Chemical characteristics	**Depth**	
	0-20 cm	**20-40 cm**
pH H_2O	5.94	5.71
P (mg dm^{-3})	36.00	5.10
K (mg dm^{-3})	165.00	66.00
Ca^{2+} (cmol$_c$ dm^{-3})	3.20	2.57
Mg^{2+} (cmol$_c$ dm^{-3})	0.75	0.48
Al^{3+} (cmol$_c$ dm^{-3})	0.00	0.00
H+Al (cmol$_c$ dm^{-3})	2.90	3.90
SB (cmol$_c$ dm^{-3})	7.51	4.37
t (cmol$_c$ dm^{-3})	7.51	4.37
T (cmol$_c$ dm^{-3})	11.41	7.27
V (%)	65.80	60.10
N (dag kg^{-1})	0.14	0.12
OM (dag kg^{-1})	3.23	2.45
P-rem (mg L^{-1})	34.00	31.60
Zn (mg dm^{-3})	4.97	1.49
Fe (mg dm^{-3})	96.20	65.20
Mn (mg dm^{-3})	89.80	49.30
Cu (mg dm^{-3})	3.22	1.68
S (mg dm^{-3})	5.40	7.80
Physical characteristics	**0-5 cm**	**5-10 cm**
Soil density (g cm^{-3})	1.10	1.01
Thick sand (g kg^{-1})	270.30	274.70
Fine sand (g kg^{-1})	244.90	239.90
Silt (g kg^{-1})	130.00	124.30
Clay (g kg^{-1})	354.80	361.10
Textural class	Clay-sandy	Clay-sandy

pH in H_2O, KCl, and $CaCl_2$ (1:2.5); P, K, Fe, Zn, Mn, Cu: extracted with Mehlich-1; Ca, Mg, Al: extracted with KCl 1 mol L^{-1}; H + Al, extracted with calcium acetate 0.5 mol L^{-1} - pH 7.0; S: extracted with monocalcium phosphate in acetic acid; SB = Exchangeable Bases Sum; t = Effective Cation Exchange Capacity; T = Cation exchange capacity at pH 7.0; V = Base Saturation Index; OM = organic matter by the Walkley & Black method (total organic carbon × 1.724). P-rem = remaining Phosphorus; N: Kjeldahl distillation. Soil Density: volumetric ring method; Sand, silt and clay: mechanical agitation and chemical dispersion with sieving and sedimentation separation (pipette method). All methods were used according to Embrapa (2011).

After transplanting, the coffee plants were fertilized using a potassium sulfate topdressing with 10 g of total K_2O per plant, divided into four applications.

During the first year after fertilization, the objective of the recommendation was to provide 15 g of N per plant (60% supplied as ammonium sulfate and 40% in the form of sunn hemp biomass). The N recovery efficiency of the fertilizers resulted in the doses of 18 g of N per plant via ammonium sulfate and 24 g of N per plant via biomass. The chemical nitrogen fertilization was split into three equal applications, while the sunn hemp residues were applied once during the final application of ammonium sulfate. However, only the first two applications of mineral fertilization were carried out before soil samples were collected. For each plant, 12 g of N was applied using mineral fertilizer between the third and fourth soil collection.

Legume production

The legume (*C. juncea*) used in the experiment was produced in a greenhouse, using 150 dm^3 boxes filled with medium sieved sand and vermiculite at a 1:1 ratio.

Sowing was performed in October at the rate of 40 seeds per linear meter and 50 cm between rows. After emergence, seedlings were thinned with an objective of 50 plants per box. The legume was produced until flowering when it was cut at 77 days after sowing. After that, the material was dried in the shade, fragmented, and then stored in raffia bags until the experiment.

Organic C and N in the soil and SOM fractions

To evaluate the response of OC and total N (TN) in the soil and in the fractions of SOM over time, soil samples were collected from the layers of 0–5 and 5–10 cm, the first sampling before the application of sunn hemp biomass and ammonium sulfate. Another set of five samples were collected every 2 months after the application of the legume and the mineral fertilizers (i.e., at 60, 120, 180, 240, and 300 days).

The samples were collected with the aid of a probe-type gauger at six different points (single samples) in each box, forming a sample composed by repetition. After each collection, the open holes were covered and marked with sticks so that no collection would be carried out at a point already sampled. The soil samples were then dried in the shade and passed through a 2 mm sieve, obtaining air-dried thin earth (ADTE). Undeformed samples were also collected at the same depths every 2 months to determine soil density using the volumetric ring method (EMBRAPA, 2011).

The free light organic matter (F-LOM) was obtained by manually stirring 5 g of soil (ADTE) and 30 mL of sodium iodide solution (NaI), with a density of 1.8 g cm^{-3} (+ 0.1 g cm^{-3}) in centrifuge tubes in triplicate. Afterwards, the samples were centrifuged for 25 min at 2520 rpm, extracting the F-LOM by filtration (SOHI et al., 2001). The granulometric fractionation was then conducted by adding 0.5 g of sodium hexametaphosphate and 200 mL of distilled water to the remaining material, which was stirred again for 16 h at 50 rpm in an orbital shaker. After stirring, the sand fraction was separated with a 0.053 mm mesh sieve, characterizing the fraction of the particulate organic matter (POM), and the rest was stored in 500 mL test tubes with distilled water. The samples were again shaken manually, and 25 mL of each sample was then pipetted, corresponding to the silt + clay fractions, considering the fraction of organic matter associated with minerals (MAM).

OC content was determined in the POM and MAM fractions, as well as the soil by wet oxidation (YEOMANS; BREMNER, 1988). N was determined by distillation using the Kjeldhal method, according to Tedesco et al. (1995). The C and N contents in the (F-LOM) fraction were determined by the difference between the soil contents and the POM and MAM fractions.

Stocks of TOC and TN, in the soil in Mg ha^{-1}, were calculated using the formula: TOC stocks or TN = content of TOC or TN (g kg^{-1}) × D_s × E/10, where D_s is the soil density at the considered depth in kg dm^{-3}, and E is the thickness of the soil layer in cm. Stocks of OC and N in the POM and MAM fractions (C-POM, C-MAM, N-POM, and N-MAM, respectively) were obtained in the same way, but considering contents of OC and N proportionally to the granulometric fractions in the soil (content of OC or N in g kg of soil^{-1}).

Statistical analysis

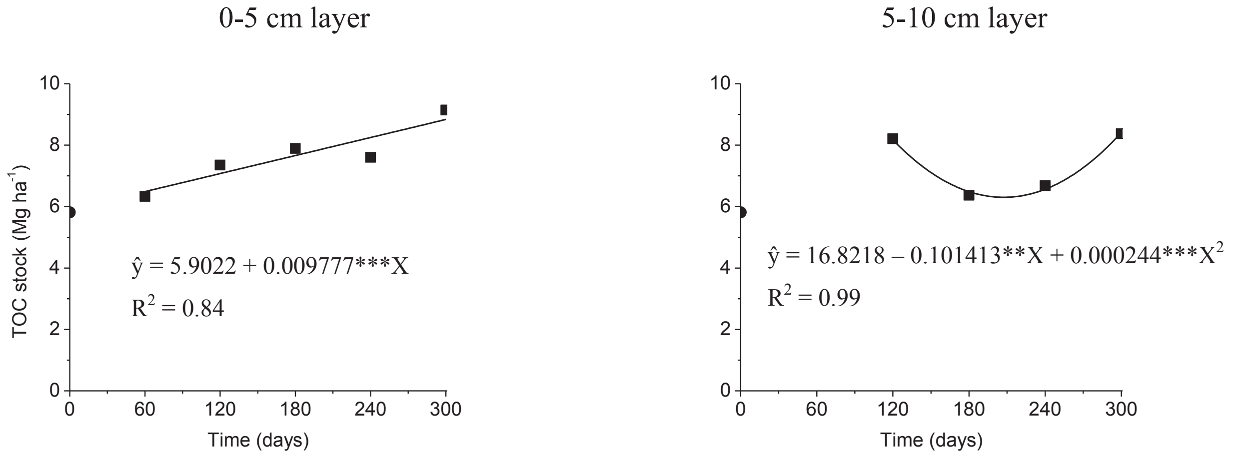
The results were subjected to analysis of variance (ANOVA) and evaluated by regression analysis ($p<0.05$). The regression model was selected based on the significance of each of the coefficients, on the value of the coefficient of determination, and its ability to explain the phenomenon in question. Sisvar software was used to aid in the analysis.

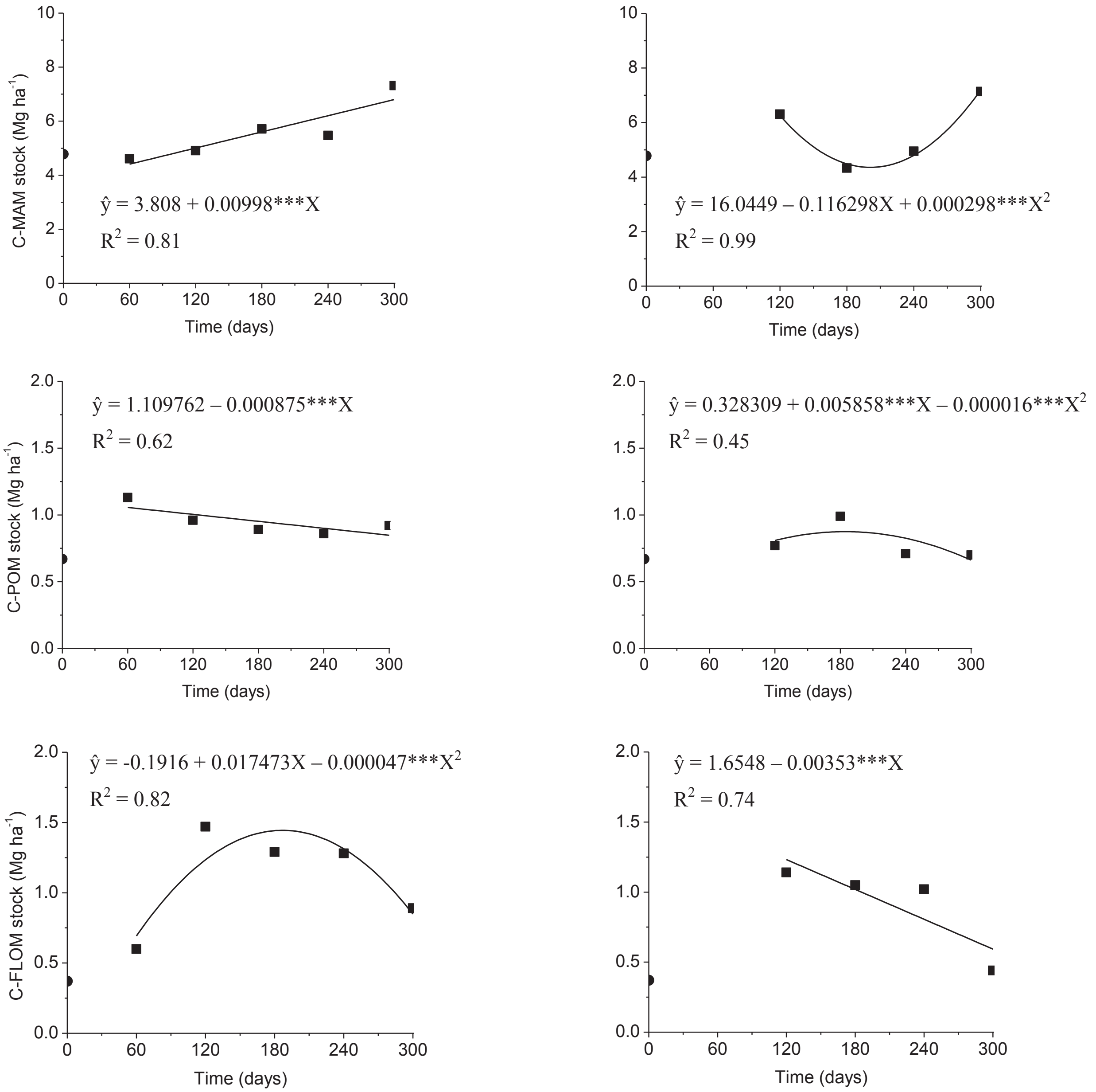
Results and Discussion

C stocks

The stocks of TOC and OC in the MAM (C-MAM) presented similar response at both depths, increasing the values despite the first reductions in the 5–10 cm layer due to the quadratic response (Figure 1). Such changes reflect the greater participation of C-MAM in TOC. On the other hand, C-MOP stocks presented a linear decreasing response, whereas C-MOLL stocks exhibited a quadratic response with increasing values until 186 days in the 0–5 cm layer (Figure 1). In the 5–10 cm layer, a quadratic response was observed in C-MOP with increasing values until 183 days, while C-MOLL stocks had a linear decreasing response (Figure 1).

Figure 1. Stocks of total organic C (TOC) in the soil and in the fractions of mineral associated (C-MAM), particulate (C-POM), and free light (C-FLOM) organic matter at 0-5 and 5-10 cm layers. *** and **: significance at 0.1 and 1%, respectively.





Despite the similar responses for TOC and C-MAM in the 0–5 cm layer, the decrease in C-MAM stocks until 60 days after fertilization was observed at time zero, contrary to the observations for TOC (Table 2). This indicated that the response observed in the soil in the first 60 d is due to the increase in C-MOP and C-MOLL stocks, as these fractions increased during this period.

Table 2. Variations (ΔStcC) in the stocks of total organic (TOC) in the soil and in the fractions of free light (C-FLOM), particulate (C-POM), and mineral associated (C-MAM) organic matter at 0-5 and 5-10 cm layers in relation to time zero

Layer	Time (days)	TOC	C-FLOM	C-POM	C-MAM	Difference[1]
		Mg ha^{-1}				
	0	5.81	0.37	0.67	4.77	0
		ΔStcC[2]				
0-5 cm	60	0.68	0.32	0.39	-0.40	0.37
	120	1.26	0.86	0.34	0.23	-0.17
	180	1.85	1.06	0.29	0.83	-0.33
	240	2.44	0.92	0.23	1.43	-0.14
	300	3.02	0.45	0.18	2.03	0.36
		TOC	C-FLOM	C-POM	C-MAM	Difference[1]
5-10 cm	120	2.35	0.86	0.14	1.61	-0.26
	180	0.66	0.65	0.20	-0.01	-0.18
	240	0.73	0.44	0.15	0.52	-0.38
	300	2.55	0.23	-0.02	3.20	-0.86

[1]Difference between TOC stocks and the sum of C stocks in each fraction. [2]Difference between C stock (TOC, C-FLOM, C-POM, or C-MAM) at different times and that at time zero.

The application of organic and mineral fertilizers may have intensified the decomposition of the original organic compartments of the soil, characterized as a priming effect (ALFAIA, 1997; KUZYAKOV, 2010). However, as the organic matter of the sunn hemp decomposes, compartment of POM and F-LOM are the first to increase because they are the compartments of the SOM that receive the contribution in their stocks as physical degradation of the organic residues (BAYER et al., 2004; MARIN et al., 2006). In addition, the POM and F-LOM are the most sensitive compartments in agriculture, compared to the MAM (BAYER et al., 2004; MARIN et al., 2006; PULROLNIK et al., 2009; FRAZÃO et al., 2010; VERGÜTZ et al., 2010), which may result in the relative reduction of C-MAM stocks, differently from what occurred for soil and other organic fractions until 60 days.

In the 0–5 cm layer, after 60 and 186 days, some reductions were observed in the C-MOP and C-MOLL stocks, respectively. These reductions might have contributed to an increase in C-MAM stocks after 60 days. It is important to note that the process is dynamic; therefore, apart from the C in POM and F-LOM, it is possible that the soluble C from the applied OM and the cycling of the soil microbiota also influenced the increases in the stocks of C-MAM (MENDONÇA et al., 2001; WOLF; WAGNER, 2005; PULROLNIK et al., 2009). In young coffee plants, the expansion and subsequent death of the roots as well as exudations in the rhizosphere (LU et al., 2003; MOTTA et al., 2006) likely contributed to the increase of C-MAM and, consequently, the TOC after 60 days in the 0–5 cm layer.

Although no incorporation of the applied OM had been found, an in-depth response was still observed. Occasionally (120 and 300 days), C-MAM stocks were relatively higher in the 5–10 cm layer—similar to TOC at 120 days. Studies have demonstrated that the addition of OM to the soil surface can lead to the rapid incorporation of C from the material into deeper layers, enriching them with OC (POIRIER et al., 2014; ISMAILI et al., 2015; GAUDER et al., 2016). Certain factors might explain the responses observed in the present work, such as the periodic irrigations conducted during the experiment, which might have promoted the decrease and stabilization of soluble forms of C in deeper layers (MENDONÇA et al., 2001; CIOTTA et al., 2004; NEU et al., 2011). Moreover, due to biological activity, the decaying material might have been transported to deeper layers primarily due to macrofaunal activity, which fragments waste and redistributes the OM in the soil (BEARE et al., 1995). However, it should also be noted that the second layer is even closer to the surface, representing a region characterized by the presence of fine roots (MOTTA et al., 2006) and high biological activity.

The changes in TOC stocks (ΔStcC) with time zero were positive in the evaluations performed in both layers (Table 2). The fractions of the SOM had positive ΔStcC in nearly all evaluations (Table 2). This is an important result, as the most labile fractions (F-LOM and POM) are the most susceptible to decomposition (BAYER et al., 2004; VERGÜTZ et al., 2010), which might contribute to short-term crop nutrition. However, the MAM fraction is more recalcitrant (BAYER et al., 2004) and drains C, contributing to the mitigation of the greenhouse effect. Additionally, the MAM fraction improves several soil properties that might contribute to higher yields (HUTCHINSON et al., 2007).

The difference observed in the last column of Table 2 is largely due to the application of the adjusted equations and the C recovery rate of the soil after the physical fractionation, which ranged from 94.92–104.18% and from 100.00–110.41% in the 0–5 and 5–10 cm layers, respectively (Table 3). These values are comparable to those obtained by Rangel and Silva (2007) when evaluating C stocks in the SOM fractions across multiple soil layers in several use and management systems.

Table 3. Stocks of total organic C (TOC) in the soil and in the fractions of free light (C-FLOM), particulate (C-POM), and mineral associated (C-MAM) organic matter at 0-5 and 5-10 cm layers at different times.

Layer	Time (days)	TOC	C-FLOM	C-POM	C-MAM	Recovery (%)[1]
		............................(Mg ha^{-1})...........................				
0-5 cm	0	5.81	0.37	0.67	4.77	100.00
	60	6.49	0.69	1.06	4.41	94.92
	120	7.08	1.23	1.01	5.01	102.40
	180	7.66	1.43	0.95	5.60	104.18
	240	8.25	1.30	0.90	6.20	101.82
	300	8.84	0.82	0.85	6.80	95.81
5-10 cm	0	5.81	0.37	0.67	4.77	100.00
	120	8.17	1.23	0.80	6.38	102.94
	180	6.47	1.02	0.86	4.77	102.78
	240	6.54	0.81	0.81	5.30	105.81
	300	8.36	0.60	0.65	7.98	110.41

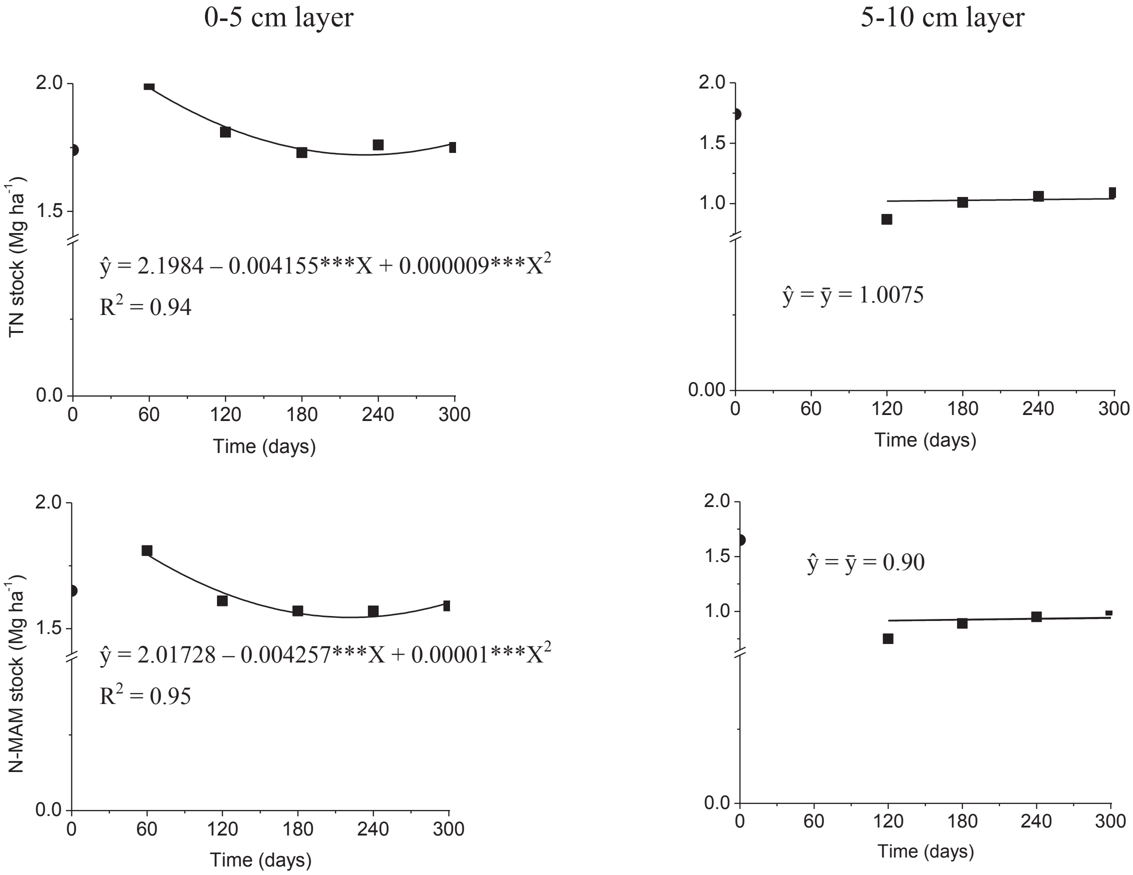
[1]Relationship between the sum of C stock in each fraction and the stock in the soil.

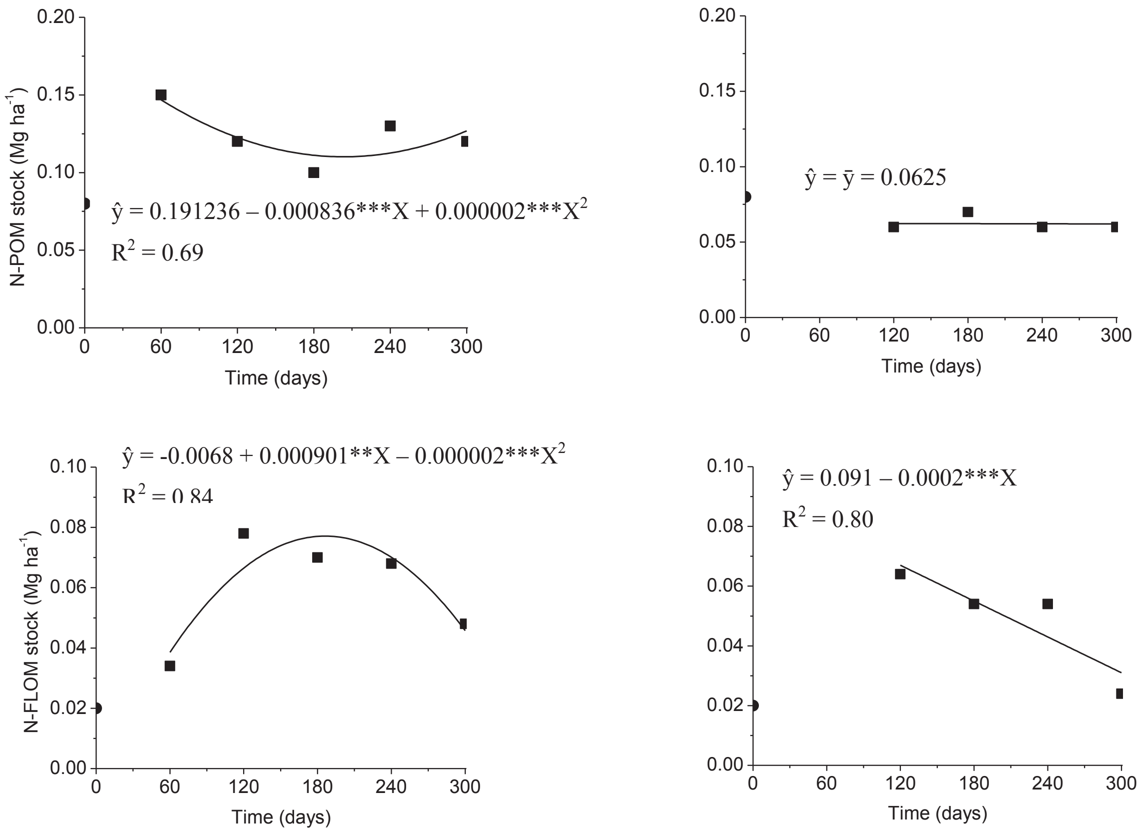
The slightly overestimated values of C in the organic fractions, relative to the TOC, observed in the present study are likely the result of the methods used for the physical fractionation of SOM and the determination of C. The use of NaI in the densimetric fractionation of SOM might have caused the overestimation in the contents of OC when it was determined by dichromatometry, compared to the results obtained by the elemental analyzer (DEMOLINARI et al., 2008). According to Demolinari et al. (2008), the overestimation of the C content was approximately 50% higher when determined by dichromatometry compared to the elemental analyzer, indicating that the procedures used in the present study were adequate and that the results demonstrated low overestimation.

N stocks

In the 0–5 cm layer, the TN stocks in the soil, MAM (N-MAM) and MOP (N-POM) exhibited a quadratic response with an initial drop in the values up to 231, 213, and 209 days, respectively (Figure 2). However, the N stocks in F-LOM (N-FLOM) presented a quadratic response in the same layer, but with an initial increase in the values up to 225 days. In the 5–10 cm layer, no significant change was detected in the TN stocks (N-POM and N-MAM) (Figure 2), while N-FLOM stocks presented a linear decreasing response (Figure 2).

Figure 2. Stocks of total N in the soil (TN) and in the fractions of free light (N-FLOM), particulate (N-POM) and associated with minerals (N-MAM) organic matter at 0-5 and 5-10 cm layers. *** and **: significance at 0.1 and 1%, respectively.





TN and N-MAM stocks showed similar responses to TOC and C-MAM, as the N associated with the most stable fractions of SOM—namely MAM—represented the highest reserves of organic N in the soil (WINCK et al., 2014). However, unlike TOC (the increments of which occurred due to the increases in C-FLOM and C-POM until 60 days), TN demonstrated similar increments to those of N-FLOM, N-POM, and N-MAM until 60 days in the 0–5 cm layer (Table 4). This result points to a differentiated dynamic between C and N caused by the fertilization. During the first 60 d, only the most labile fractions enriched themselves with C; in the case of N, an incorporation in soil was also found in the most recalcitrant fraction (MAM). Such a process may result in enhanced soil properties due to the increase in OC. However, the incorporation of N in MAM initially reduces short-term crop nutrition possibilities. The variations in N stocks of the MAM fraction became smaller over time, and later, they were negative in relation to time zero, indicating the mineralization of N in this compartment (Table 4).

Table 4. Variations in N stocks (ΔStcN) in the soil (TN) and in the fractions of free light (N-FLOM), particulate (N-POM), and mineral associated (N-MAM) organic matter at 0-5 and 5-10 cm layers in relation to time zero.

Layer	Time (days)	TN	N-FLOM	N-POM	N-MAM	Difference[1]
		....	....	Mg ha^{-1}	....	....
	0	1.74	0.02	0.08	1.65	0
				ΔStcN[2]		
0-5 cm	60	0.24	0.03	0.07	0.15	-0.01
	120	0.09	0.07	0.04	0.01	-0.03
	180	0.0	0.08	0.03	-0.07	-0.04
	240	-0.02	0.09	0.03	-0.07	-0.03
	300	0.02	0.08	0.04	-0.01	-0.09
		TN [ns]	**N-FLOM**	**N-POM** [ns]	**N-MAM** [ns]	**Difference**[1]
5-10 cm	120	-0.74	0.05	-0.14	-0.75	0.10
	180	-0.74	0.04	-0.14	-0.75	0.11
	240	-0.74	0.02	-0.14	-0.75	0.13
	300	-0.74	0.01	-0.14	-0.75	0.14

[1]Difference between TN stock and the sum of N stocks in each fraction.[2]Difference between N stock (TN, N-FLOM, N-POM and N-MAM) at different times and at that in time zero. [ns]Non-significant (F test, $p \geq 0.05$).

The drop in the values of TN, N-MAM, and N-POM stocks in the 0–5 cm layer after 60 days indicates N mineralization of the MAM and POM compartments, with subsequent absorption by the coffee plants and/or leaching to deeper layers and/or losses by volatilization. At the same time, it is likely that mineralization of the N associated with the F-LOM compartment occurred—although the response indicates an increment in the stock of that compartment. This might occur due to the decomposition of the applied OM and the growth of the coffee roots, which contribute to the F-LOM.

Stocks of N-MAM and N-POM exhibited small increments after 213 and 209 days, respectively. In the MAM fraction, these increases might be the result of the first post-planting N fertilization, mineralization of some POM (although increases in its N stocks had occurred), and mineralization of the F-LOM fraction and most recalcitrant OM in the soil. The mineralization observed in the F-LOM fraction, as well as the decomposition and mineralization of the OM deposited on the soil might have been influenced by the addition of mineral N during the first post-planting fertilization due to the priming effect. The consequence of this event was the increase in the N-POM stock.

The N added to the soil in the form of mineral and organic fertilizer promoted an increase of 0.24 Mg ha^{-1} of N in the 0–5 cm layer until 60 days. Of this total, 0.03 and 0.07 Mg ha^{-1} were associated with F-LOM and POM, respectively, while the rest was associated with MAM (Table 4). In this case, approximately 60% of the N added to the soil was drawn to the SOM stable fractions, indicating a rapid stabilization of this nutrient in the most recalcitrant organic compartments. Studies conducted in temperate climates have shown that there was more N in the 0–10 cm layer (due to the litter decomposing on the soil) in the organo-mineral associations of silt and clay than in other organic fractions, after 140 days (BIMÜLLER et al., 2013). The highest and the fastest incorporation of N in MAM is directly related to lower yields (KÖLBL et al., 2006) if its later mineralization is not synchronized with the crop demand.

This early difference in the dynamics of N and C may partially explain the lack of increased productivity and/or growth variables of coffee trees provided green manure, despite the positive responses on soil properties reported in some studies (BERGO et al., 2006; PAULO et al., 2006; PARTELLI et al., 2011; MOREIRA et al., 2014). However, it is important to emphasize that, in field conditions, several factors affect the response of coffee to green manure—especially those related to intercropping.

The results of the present study may be partially explained by certain factors. For instance, the application of organic and mineral sources of N might have promoted the rapid stabilization of N in MAM (MORAN et al., 2005). The conditions under which the work was conducted—in a protected environment with relatively low temperature variations, besides the periodic irrigations that aimed to allow the soil to reach field capacity and to supply C and N sources -might have also promoted the greater biological activity by accelerating the transformation of the applied OM as well as the incorporation of its nutrients in the MAM fraction.

Despite the reductions in the stocks of NT, N-MAM, and N-POM after 60 days, the variations of these N stocks (ΔStcN) in the 0–5 cm layer in relation to time zero were positive the majority of the time, indicating the effect of N fertilization (Table 4). The changes in TN stocks in the 0–5 cm layer were positive during the first 180 days. At 240 days, the values were negative and then became positive again. It is certain that the response after 240 days was due to the application of part of the first-year post-planting N fertilization when 12 g box^{-1} of N was supplied in the form of ammonium sulfate. In the same layer, the ΔStcN in the F-LOM and POM fractions remained positive throughout the evaluations, while the ΔStcN in the MAM fraction became negative after 180 days, in relation to time zero.

No effect was found in the N-POM and N-MAM stocks in the 5–10 cm layer, which was reflected by the TN. Once the value of the means of each fraction and the soil were lower than those of time zero, ΔStcN was consistently negative in the MAM and POM fractions and the soil (Table 4). This points to the mineralization of the N from these organic compartments and the output of N from this layer, which may be due to leaching and absorption by the coffee plants. In the 5–10 cm layer, only the F-LOM fraction presented a positive ΔStcN during the evaluated period.

The differentiated behavior of the N stocks between the layers can be explained since—in the most superficial layer, even with the occurrence of mineralization of the organic compartments—the N absorption by coffee plants and the potential leaching (excluding the losses by volatilization) caused a constant decomposition and mineralization of the OM from the *Crotalaria*, which was a major determinant of the results.

It is possible to conclude that fertilization with *C. juncea* increased C stocks in labile and recalcitrant fractions of SOM. Additionally, under the conditions of this experiment, the majority of the N supplied to the soil was rapidly incorporated into the most stable fraction of SOM, and this might can reduced the efficiency of the green manuring.

Further studies using a shorter time interval between fertilization and the first soil sampling should be able to demonstrate how quickly N is incorporated into MAM and subsequently mineralized.

References

ALFAIA, S. S. Mineralização do nitrogênio incorporado como material vegetal em três solos da Amazônia central. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 21, n. 3, p. 387-392, 1997.

AMBROSANO, E. J.; TRIVELIN, P. C. O.; CANTARELLA, H.; AMBROSANDO, G. M. B.; SCHAMMASS, E. A.; MURAOKA, T.; ROSSI, F. ^{15}N-labeled nitrogen from green manure and ammonium sulfate utilization by the sugarcane ratoon. *Scientia Agricola*, Piracicaba, v. 68, n. 3, p. 361-368, 2011.

ARAÚJO, A. S. F.; TEIXEIRA, G. M.; CAMPOS, A. X.; SILVA, F. C. Utilização de nitrogênio pelo trigo cultivado em solo fertilizado com adubo verde (C*rotalaria juncea*) e/ou ureia. *Ciência Rural*, Santa Maria, v. 35, n. 2, p. 284-289, 2005.

ARAÚJO, E. S.; GUERRA, J. G. M.; ESPINDOLA, J. A. A.; URQUIAGA, S.; BODDEY, R. M.; MARTELLETO, L. A. P.; ALVES, B. J. R. Recuperação no sistema solo-planta de nitrogênio derivado da adubação verde aplicada à cultura do repolho. *Pesquisa Agropecuária Brasileira*, Brasília, v. 46, n. 7, p. 729-735, 2011.

ARAÚJO, J. B. S.; RODRIGUES, L. B.; RODRIGUES, M. C.; MARTINEZ, H. E. P.; SANTOS, R. H. S. Adubação nitrogenada em cafeeiros com biomassa de feijão-de-porco. *Coffee Science*, Lavras, v. 9, n. 3, p. 336-346, 2014.

BAYER, C.; MARTIN-NETO, L.; MIELNICZUK, J.; PAVINATO, A. Armazenamento de carbono em frações lábeis da matéria orgânica de um Latossolo Vermelho sob plantio direto. *Pesquisa Agropecuária Brasileira*, Brasília, v. 39, n. 7, p. 677-683, 2004.

BEARE, M. H.; COLEMAN, D. C.; CROSSLEY, D. A.; HENDRIX, P. F.; ODUM, E. P. A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant and Soil*, The Hague, v. 170, n. 1, p. 5-22, 1995.

BERGO, C. L.; PACHECO, E. P.; MENDONÇA, H. A.; MARINHO, J. T. S. Avaliação de espécies leguminosas na formação de cafezais no segmento da agricultura familiar no Acre. *Acta Amazonica,* v. 36, n. 1, p. 19-24, 2006.

BIMÜLLER, C.; NAUMANN, P. S.; BUEGGER, F.; DANNENMANN, M.; ZELLER, B.; VON LÜTZOWA, M.; KÖGEL-KNABNER, I. Rapid transfer of ^{15}N from labeled beech leaf litter to functional soil organic matter fractions in a Rendzic Leptosol. *Soil Biology & Biochemistry*, Oxford, v. 58, n. 1, p. 323-331, 2013.

CHRISTENSEN, B. T. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *European Journal of Soil Science*, Weinheim, v. 52, n. 3, p. 345-353, 2001.

CIOTTA, M. N.; BAYER, C.; ERNANI, P. R.; FONTOURA, S. M. V.; WOBETO, C.; ALBUQUERQUE, J. A. Manejo da calagem e os componentes da acidez de latossolo bruno em plantio direto. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 28, n. 2, p. 317-326, 2004.

DELARMELINDA, E. A.; SAMPAIO, F. A. R.; DIAS, J. R. M.; TAVELLA, L. B.; SILVA, J. S. Adubação verde e alterações nas características químicas de um Cambissolo na região de Ji-Paraná-RO. *Acta Amazonica*, Manaus, v. 40, n. 3, p. 625-628, 2010.

DEMOLINARI, M. S. M.; SILVA, I. R.; LIMA, A. M. N.; VERGÜTZ, L.; MENDONÇA, E. S. Efeito da solução de separação densimétrica na quantidade e qualidade da matéria orgânica leve e na quantificação do carbono orgânico da fração pesada. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 32, n. 2, p. 871-879, 2008.

DINIZ, E. R.; VARGAS, T. O.; SANTOS, R. H. S.; ALMEIDA, A. R.; MATTOS, U. J. B. M. Crescimento e produção de brócolis adubado com doses mucuna-cinza em casa de vegetação. *Semina: Ciências Agrárias*, Londrina, v. 36, n. 3, p. 1277-1286, 2015.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Manual de métodos de análise de solo. 2. ed. Rio de Janeiro: EMBRAPA, 2011. 230 p.

EUROPEAN FERTILIZER MANUFACTURERS' ASSOCIATION - EFMA. Best available techniques for pollution prevention and control in the European fertilizer industry – production of Ammonia. Brussels: EFMA, 2000. 44 p.

FRAZÃO, L. A.; SANTANA, I. K. S.; CAMPOS, D. V. B.; FEIGL, B. J.; CERRI, C. C. Estoques de carbono e nitrogênio e fração leve da matéria orgânica em Neossolo Quartzarênico sob uso agrícola. *Pesquisa Agropecuária Brasileira*, Brasília, v. 45, n. 10, p. 1198-1204, 2010.

GAUDER, M.; BILLEN, N.; ZIKELI, S.; LAUB, M.; GRAEFF-HÖNNINGER, S.; CLAUPEIN, W. Soil carbon stocks in different bioenergy cropping systems including subsoil. *Soil & Tillage Research*, Amsterdam, v. 155, n. 1, p. 308-317, 2016.

GUIMARÃES, P. T. G.; GARCIA, A. W. R.; ALVAREZ, V. H.; PREZOTTI, L. C.; VIANA, A. S.; MIGUEL, A. E.; MALAVOLTA E.; CORRÊA, J. B.; LOPES, A. S.; NOGUEIRA, F. D.; MONTEIRO, A. V. O.; OLIVEIRA, J. A. Cafeeiro. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ, V. V. H. (Ed.). *Recomendações para o uso de corretivos e fertilizantes em Minas Gerais*. 5ª Aproximação. Viçosa, MG: CSFSEMG/UFV, 1999. p. 289-302.

HUTCHINSON, J. J.; CAMPBELL, C. A.; DESJARDINS, R. L. Some perspectives on carbon sequestration in agriculture. *Agricultural and Forest Meteorology*, Amsterdam, v. 142, n. 2-4, p. 288-302, 2007.

ISMAILI, K.; ISMAILI, M.; IBIJBIJEN, J. The use of ^{13}C and ^{15}N based isotopic techniques for assessing soil C and N changes under conservation agriculture. *European Journal of Agronomy*, Montpellier v. 64, n. 1,

p. 1-7, 2015.

KÖLBL, A.; VON LÜTZOW, M.; KÖGEL-KNABNER, I. Decomposition and distribution of ^{15}N labelled mustard litter (*Sinapis alba*) in physical soil fractions of a cropland with high- and low-yield field areas. *Soil Biology and Biochemistry*, Amsterdam, v. 38, n. 11, p. 3292-3302, 2006.

KUZYAKOV, Y. Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry*, Amsterdam, v. 42, n. 9, p. 1363-1371, 2010.

LANGE, A.; BOLOGNA, I. R.; FARONI, C. E.; TRIVELIN, P. C. O. Aproveitamento pelo trigo do nitrogênio residual da crotalária (C*rotalaria juncea*) e da ureia aplicado ao solo em cultivo precedente. *Ciência Rural*, Santa Maria, v. 39, n. 6, p. 1715-1720, 2009.

LOVATO, T.; MIELNICZUK, J.; BAYER, C.; VEZZANI, F. Adição de carbono e nitrogênio e sua relação com os estoques no solo e com o rendimento do milho em sistemas de manejo. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 28, n. 1, p. 175-187, 2004.

LU, Y.; WATANABE, A.; KIMURA, M. Carbon dynamics of rhizodeposits, root- and shoot-residues in a rice soil. *Soil Biology and Biochemistry*, Amsterdam, v. 35, n. 9, p. 1223-1230, 2003.

MARIN, A. M. P.; MENEZES, R. S. C.; SILVA, E. D.; SAMPAIO, E. V. S. B. Efeito da *Gliricidia sepium* sobre nutrientes do solo, microclima e produtividade do milho em sistema agroflorestal no agreste paraibano. *Revista Brasileira de Ciências Solo*, Viçosa, MG, v. 30, n. 3, p. 555-564, 2006.

MENDONÇA, E. S.; SILVA, A. C.; MEDEIROS, M. L.; FONTES, M. P. F. Carbono orgánico extraído por soluciones salinas y su relación com otras formas de carbono de suelos tropicales. *Agrociência*, Texcoco, v. 35, n. 4, p. 397-406, 2001.

MIRANDA, F. R.; SANTANA, M. G. S.; SOUZA, C. C. M.; OLIVEIRA, C. H. C. Calibração do sensor dielétrico ECH_2O em dois tipos de solos. *Revista Ciência Agronômica*, Fortaleza, v. 38, n. 3, p. 317-321, 2007.

MORAN, K. K.; SIX, J.; HORWATH, W. R.; van KESSEL, C. Role of mineral-nitrogen in residue decomposition and stable soil organic matter formation. *Soil Science Society of America Journal*, Madison, v. 69, n. 6, p. 1730-1736, 2005.

MOREIRA, G. M.; BARRELLA, T. P.; MARTINS NETO, F. L.; MARTINEZ, H. E. P.; SANTOS, R. H. S. Consorciação em cafeeiros orgânicos por diferentes períodos com feijão-de-porco ou lablabe. *Coffee Science*, Lavras, v. 9, n. 4, p. 456-464, 2014.

MOTTA, A. C. V.; NICK, J. A.; YORINORI, G. T.; SERRAT, B. M. Distribuição horizontal e vertical da fertilidade do solo e das raízes de cafeeiro (*Coffea arabica* L.) cultivar Catuaí. *Acta Scientiarum Agronomy*, Maringá, v. 28, n. 4, p. 455-463, 2006.

MYROLD, D. D. Transformations of nitrogen. In: SYLVIA, D. M.; FUHRMANN, J. J.; HARTEL, P. G.; ZUBERER, D. A. (Ed.). *Principles and applications of soil microbiology*. New Jersey: Pearson Prentice Hall, 2005. p. 333-372.

NEU, V.; NEILL, C.; KRUSCHE, A. V. Gaseous and fluvial carbon export from an Amazon forest watershed. *Biogeochemistry*, Coverage, v. 105, n. 1-3, p. 133-147, 2011.

PARTELLI, F. L.; VIEIRA, H. D.; FERREIRA, E. P. B.; VIANA, A. P.; ESPINDOLA, J. A. A.; URGUIAGA, S.; BODDEY, R. M. Biologic dinitrogen fixation and nutrient cycling in cover crops and their effect on organic Conilon coffee. *Semina: Ciências Agrárias*, Londrina, v. 32, n. 3, p. 995-1006, 2011.

PAULO, E. M.; BERTON, R. S.; BULISANI, E. A.; KASAI, F. S. Produtividade do cafeeiro Mundo Novo enxertado e submetido à adubação verde antes e após recepa da lavoura. *Bragantia*, v. 65, n. 1, p .115-120, 2006.

PERIN, A.; SANTOS, R. H. S.; CABALLERO, S. S. U.; GUERRA, J. G. M.; GUSMÃO, L. A. Acúmulo e liberação de P, K, Ca e Mg em crotalária e milheto solteiros e consorciados. *Revista Ceres*, Viçosa, MG, v. 57, n. 2, p. 274-281, 2010.

POIRIER, V.; ANGERS, D. A.; WHALEN, J. K. Formation of millimetric-scale aggregates and associated retention of ^{13}C e ^{15}N-labelled residues are greater in subsoil than topsoil. *Soil Biology & Biochemistry*, Amsterdam, v. 75, n. 1, p. 45-53, 2014.

PULROLNIK, K.; BARROS, N. F.; SILVA, I. R.; NOVAIS, R. F.; BRANDANI, C. B. Estoques de carbono e nitrogênio em frações lábeis e estáveis da matéria orgânica de solos sob eucalipto, pastagem e cerrado no Vale do Jequitinhonha-MG. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 33, n. 5, p. 1125-1136, 2009.

RANGEL, O. J. P.; SILVA, C. A. Estoques de carbono e nitrogênio e frações orgânicas de latossolo submetido a diferentes sistemas de uso e manejo. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 31, n. 6, p. 1609-1623, 2007.

RIBEIRO, P. H.; SANTOS, J. V. V. M.; COSER, S. M.; NOGUEIRA, N. O.; MARTINS, C. A. S. Adubação verde, os estoques de carbono e nitrogênio e a qualidade da matéria orgânica do solo. *Revista Verde*, Pombal, v. 6, n. 1, p. 43-50, 2011.

RICCI, M. S. F.; ALVES, B. J. R.; MIRANDA, S. C.; OLIVEIRA, F. F. Growth rate and nutritional status of an organic coffee cropping system. *Scientia Agricola*, Piracicaba, v. 62, n. 2, p. 138-144, 2005.

SEO, J.; MEISINGER, J. J.; LEE, H. Recovery of Nitrogen-15–Labeled hairy vetch and fertilizer applied to corn. *Agronomy Journal*, Madison, v. 98, n. 2, p. 245-254, 2006.

SOHI, S. P.; MAHIEU, N.; ARAH, J. R. M.; POWLSON, D. S.; MADARI, B.; GAUNT, J. L. A procedure for isolating soil organic matter fractions suitable for modeling. *Soil Science Society of America Journal*, Madison, v. 65, n. 4, p. 1121-1128, 2001.

SOUZA, E. C.; CARNEIRO, M. A. C.; PAULINO, H. B.; SILVA, C. A.; BUZETTI, S. Alterações nas frações do carbono em um neossolo quartzarênico submetido a diferentes sistemas de uso do solo. *Acta Scientiarum Agronomy*, Maringá, v. 28, n. 3, p. 305-311, 2006.

TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J. *Análise de solo, plantas e outros materiais*. 2. ed. Porto Alegre: Departamento de Solos da Universidade Federal do Rio Grande do Sul, 1995. 174 p.

VERGÜTZ, L.; NOVAIS, R. F.; SILVA, I. R.; BARROS, N. F.; NUNES, T. N.; PIAU, A. A. M. Mudanças na matéria orgânica do solo causadas pelo tempo de adoção de um sistema agrossilvopastoril com eucalipto. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 34, n. 1, p. 43-57, 2010.

WINCK, B. R.; VEZZANI, F. M.; DIECKOW, J.; FAVARETTO, N.; MOLIN, R. Carbono e nitrogênio nas frações granulométricas da matéria orgânica do solo, em sistemas de culturas sob plantio direto. *Revista Brasileira de Ciência do Solo*, Viçosa, MG, v. 38, n. 3, p. 980-989, 2014.

WOLF, D. C.; WAGNER, G. H. Carbon transformations and soil organic matter formation. In: SYLVIA, D. M.; FUHRMANN, J. J.; HARTEL, P. G.; ZUBERER, D. A. (Ed.). *Principles and applications of soil microbiology*. New Jersey: Pearson Prentice Hall, 2005. p. 285-332.

YEOMANS, J. C.; BREMNER, J. M. A. Rapid and precise method for routine determination of organic carbon in soil. *Communications in Soil Science and Plant Analysis*, New York, v. 19, n. 13, p. 1467-1476, 1988.