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Biological Nitrogen Fixation by Legumes and N Uptake by Coffee Plants

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ABSTRACT: Green manures are an alternative for substituting or supplementing mineral nitrogen fertilizers. The aim of this study was to quantify biological N fixation (BNF) and the N contribution derived from BNF (N-BNF) to N levels in leaves of coffee intercropped with legumes grown on four family farms located in the mountainous region of the Atlantic Forest Biome in the state of Minas Gerais, Brazil. The following green manures were evaluated: pinto peanuts (*Arachis pintoi*), calopo (*Calopogonium mucunoides*), crotalaria (*Crotalaria spectabilis*), Brazilian stylo (*Stylosanthes guianensis*), pigeon pea (*Cajanus cajan*), lablab beans (*Dolichos lablab*), and velvet beans (*Stizolobium deeringianum*), and spontaneous plants. The experimental design was randomized blocks with a 4 × 8 factorial arrangement (four agricultural properties and eight green manures), and four replications. One hundred grams of fresh matter of each green manure plant were dried in an oven to obtain the dry matter. We then performed chemical and biochemical characterizations and determined the levels of ^{15}N and ^{14}N, which were used to quantify BNF through the ^{15}N ($\delta^{15}N$) natural abundance technique. The legumes *C. mucunoides, S. guianensis, C. cajan*, and *D. lablab* had the highest rates of BNF, at 46.1, 45.9, 44.4, and 42.9 %, respectively. *C. cajan* was the legume that contributed the largest amount of N (44.42 kg ha^{-1}) via BNF.*C. cajan, C. spectabilis*, and *C. mucunoides* transferred 55.8, 48.8, and 48.1 %, respectively, of the N from biological fixation to the coffee plants. The use of legumes intercropped with coffee plants is important in supplying N, as well as in transferring N derived from BNF to nutrition of the coffee plants.

Keywords: family farming, green manure, coffee growing, ^{15}N.

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INTRODUCTION

In the context of climate change, the Brazilian government has pledged to reduce greenhouse gas (GHG) emissions by between 36.1 to 38.9 %, based on emissions projected for 2020 (3236 million tons of equivalent CO_2). With respect to the agricultural sector, the expectation is to reduce emissions by 730 million tons of equivalent CO_2 by 2020. One of the actions involved in this is expansion of biological N fixation (BNF) to 5.5 million hectares of cultivated land to replace the use of N fertilizers (Brasil, 2010). However, for crops in which it is not possible to totally depend on BNF, the use of legumes is an alternative for substituting or supplementing mineral N fertilization (Ambrosano et al., 2005).

Considering that the estimates for production, distribution, and application of 1 kg of N-fertilizer correspond to 4.5 kg of equivalent CO_2 emitted into the atmosphere (Oliveira et al., 2014), BNF has considerable potential for wider use in Brazilian agriculture. Additionally, the low recovery of the N in the fertilizers – around 50-60 % (Cantarella, 2007) – highlights the need to seek alternatives that enable the use of local inputs without affecting agricultural production (Perez et al., 2004). Thus, the use of legumes that can efficiently facilitate BNF may contribute to the economic viability and sustainability of coffee production systems by reducing the need for use of synthetic N (Brito et al., 2009; Guimarães et al., 2016).

In studies on N dynamics in the soil-plant system using the difference method, it is difficult to identify the amount of N recovered from the source and the origin of the source used (Brito et al., 2009). However, using a source marked with ^{15}N, as in the ^{15}N ($\delta^{15}N$) natural abundance technique, it is possible to more accurately quantify N retention. Estimates of BNF in the field are highly variable, accounting for 40-90 % of the total N accumulated by the fixing crop (Rumjanek et al., 2005). This variability can be attributed to differences in the plant species, the plant genotypes, and the rhizobia, as well as the soil and climatic conditions (Martins et al., 2003).

Assessing the BNF contribution to accumulation of N in crotalaria (*Crotalaria juncea*) and in the intercropping of crotalaria and pearl millet (*Pennisetum americanum*), Perin et al. (2004) reported $\delta^{15}N$ values of 9.32, 3.43, and 3.02 ‰ for spontaneous plants (non-fixing crops), crotalaria alone, and intercropped crotalaria, respectively. Crotalaria alone had 57 % of N derived from BNF, which corresponded to 173.2 kg ha^{-1} of N; whereas in intercropped crotalaria, 61 % of N was derived from BNF, which corresponds to 89.1 kg ha^{-1} of N.

Using the same $\delta^{15}N$ technique in leguminous trees intercropped with *Eucalyptus grandis*, Coelho et al. (2007) verified a high contribution of N derived from BNF: 92, 74, and 74 % for *Mimosa scabrella*, *Mimosa caesalpiniaefolia*, and *Inga* sp, respectively. For the leguminous Asian shrub Flemingia (*Flemingia macrophylla*), 76 % of the N taken up was derived from BNF, which represented 57 kg ha^{-1} of N in the aerial part, 360 days after planting the seedlings (Salmi et al., 2013).

The BNF contributed 80 % of the N accumulated in the aerial part of gliricidia (*Gliricidia sepium*), which corresponded to 109 kg ha^{-1}, and 64.5 % of the N for crotalaria (*Crotalaria juncea*), which corresponded to 96.5 kg ha^{-1}, when intercropped with soursop and mango trees (Paulino et al., 2009). These authors reported that crotalaria and gliricidia transferred 22.5 and 40 % of the N from BNF, respectively , to the soursop trees. In cultivation of *nanicão* banana, estimations of BNF from tropical leguminous kudzu (*Pueraria phaseoloides*), pinto peanuts (*Arachis pintoi*), and siratro (*Macroptilium atropurpureum*) were 86.2 % (305.5 kg ha^{-1}), 66.9 % (201.6 kg ha^{-1}), and 38.2 % (89.3 kg ha^{-1}), respectively; while 33.7 % (kudzu), 40.5 % (pinto peanuts), and 24.2 % (siratro) of the N found in the leaf tissue of the banana plants was from BNF (Espindola et al., 2006).

Coffee is one of the most demanding crops in terms of N – the recommendation ranges from 150 to 450 kg ha^{-1} of N, depending on the age of the crop and yield expectations (Guimarães et al., 1999; Schiavinatti et al., 2011). We are unaware of results from research utilizing the $\delta^{15}N$ technique to demonstrate the contribution of BNF from legumes in nutrition of coffee plants. With the hypothesis that green manures contribute to N nutrition of coffee, the aim of this study was to quantify the contribution of BNF in N fertilization and in the levels of the leaves of coffee intercropped with legumes cultivated by family farmers located in the mountainous region of the Atlantic Forest Biome, Brazil.

MATERIALS AND METHODS

The experiments were set up in January 2004 and conducted until December 2007 in four experimental units on family farms located in the mountainous region of the Atlantic Forest Biome, state of Minas Gerais, Brazil. The experimental units were set up in two areas of family farms in the municipality of Araponga (Araponga 1 and Araponga 2) – one in Eugenópolis and the other in Pedra Dourada. The geographical coordinates ranged from 20° 41’ S to 20° 50’ S and 42° 08’ W to 42° 33’ W, at altitudes from 600 to 1,100 m above sea level; average temperatures were from 14.4 to 19.2 °C.

The landscapes of the experimental units are hilly, with slopes between 20 and 45 %. The soils are well drained and deep. They are also highly weathered and acidic and have low natural fertility. The soils of the four study areas were Oxisols (Soil Survey Staff, 2010), corresponding to *Latossolo Vermelho Amarelo* in the Brazilian classification system (Embrapa, 2006). The chemical and physical characterizations for the depth of 0.0-0.2 m, which were performed at the time the experiments were set up, are shown in table 1.

The coffee fields had been managed in an organic system since seedling formation. Coffee seedlings were planted in November/December 2002, but before planting, the soil was corrected with limestone, and thermophosphate and potassium sulfate were applied, in accordance with soil analysis and the recommendations of the Soil Fertility Commission for the State of Minas Gerais (Alvarez V et al., 1999).

Table 1. Chemical and physical properties at the depth of 0.00-0.2 m of the soils before setting up the experiments

Property	Araponga 1	Araponga 2	Pedra Dourada	Eugenópolis
pH(H_2O) (1:2.5)	5.24	5.42	5.04	5.16
P (mg dm^{-3})	1.00	1.28	2.92	2.05
K (mg dm^{-3})	59.80	56.75	53.50	51.45
Al^{3+} ($cmol_c$ dm^{-3})	0.47	0.53	0.59	0.56
Ca^{2+} ($cmol_c$ dm^{-3})	1.74	1.17	0.99	1.03
Mg^{2+} ($cmol_c$ dm^{-3})	0.74	0.69	0.47	0.54
OC (g kg^{-1})	29.04	31.10	36.80	35.15
Zn (mg dm^{-3})	1.17	1.22	1.56	1.47
Fe (mg dm^{-3})	40.7	32.15	14.70	18.52
Mn (mg dm^{-3})	10.4	13.08	20.20	22.34
Cu (mg dm^{-3})	0.50	0.48	0.38	0.41
Sand (g kg^{-1})	390	330	360	340
Clay (g kg^{-1})	520	550	450	480
Textural class	Clayey	Clayey	Clayey	Clayey

P, K, Zn, Fe, Mn, Cu: extractor Mehlich-1; Al, Ca, Mg: extractor 1 mol L^{-1} KCl; OC: organic carbon, Walkley-Black method; sand and clay: pipette method.

At each location, the Catuai Vermelho cultivar of *Coffea arabica* was cultivated at a spacing of 2.8 × 0.5 m. Over the course of 2004, three applications of 150 g per plant of castor bean cake, one 60 g m^{-2} application of potassium sulfate, and one 150 g m^{-2} application of limestone were administered within and between the coffee rows. In 2005, 120 g m^{-2} of limestone, 80 g m^{-2} of thermophosphate, and 20 g m^{-2} of potassium sulfate were applied between the rows; within the rows, 400 g per plant of castor bean cake was divided into four applications throughout the rainy season, as well as 200 g m^{-2} of potassium sulfate. In 2006 and 2007, 120 g m^{-2} of limestone, 80 g m^{-2} of thermophosphate, and 20 g m^{-2} of potassium sulfate were applied between the rows; while within the rows, 750 g per plant of castor bean cake was divided into three applications, as well as 200 g m^{-2} of potassium sulfate.

The experimental design was randomized blocks, with four replications, in a 4 × 8 factorial arrangement: four locations and eight different types of green manure were evaluated between the coffee rows, which included pinto peanuts (*Arachis pintoi*), calopo (*Calopogonium mucunoides*), (*Crotalaria spectabilis*), Brazilian stylo (*Stylosanthes guianensis*), pigeon peas (*Cajanus cajan*), lablab beans (*Dolichos lablab*), velvet beans (*Stizolobium deeringianum*), and spontaneous plants.

The legumes were planted from 2004 to 2007, always at the beginning of the rainy season, cut 150 days after sowing, and then spread under the canopy of the coffee plants. The legumes were sown between the coffee rows at a spacing of 0.4 m, which made for a total of five rows of legumes between each coffee row. After germination, plants were thinned to five per linear meter, which corresponded to 89,286 plants per hectare.

In 2007, 150 days after seeding, the aerial part of the plant was cut and weighed. After obtaining fresh matter weight (data not shown), about 100 g of this fresh matter was oven dried at 65 °C until reaching constant weight, obtaining dry matter. Subsequently, the materials were ground and passed through a 2 mm sieve. This material was used for evaluation of the BNF rate.

For characterization of the green manures, the total C and N content obtained by dry combustion was determined with a Perkin Elmer CHNS/O 2400 analyzer. Elementary P was determined according to Braga and Defelipo (1974) after nitric perchloric digestion (Sarruge and Haag, 1974). In the same digestion, K content was determined by flame photometry, and Ca and Mg were determined by atomic absorption spectrophotometry. The soluble polyphenols were extracted with methanol (50 %) and determined by colorimetry, using the Folin-Denis reagent (Anderson and Ingram, 1996). The cell wall components were obtained by the serial method (van Soest et al., 1991) using 2 mL of a 1 % amylase solution per sample in determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF). For the material analyzed, the hemicellulose values were determined (as a percentage of dry matter) by subtracting the ADF from the NDF. The cellulose levels were obtained by subtracting the lignin from the ADF.

The legumes and the spontaneous plants cultivated at the locations studied had similar chemical composition and biochemistry. The average characterization of the chemical and biochemical compositions of the residues studied can be seen in table 2.

Leaf samples of the coffee plants were taken when the coffee berries had not yet fully matured. In each experimental unit, 24 leaves from the upper middle third of the coffee plant were collected. The isotopic composition of the samples of the green manures and the leaves of the coffee plants was determined using a C and N autoanalyzer coupled to a SerCon 20-20 mass spectrometer.

The N_2 fixation rates were calculated using the equation proposed by Shearer and Kohl (1986). In general, the differences are calculated between the $\delta^{15}N$ of the fixing plants (which in this case are the legumes) and the $\delta^{15}N$ of the non-fixing plants (the spontaneous

Table 2. Chemical and biochemical contents of the green manures and spontaneous plants on the four farms located in the Zona da Mata region, state of Minas Gerais, Brazil

Green manure	C	N	P	K	Ca	Mg	HM	CL	LG	PP	C/P	C/N
	dag kg^{-1}											
A. pintoi	42.70	2.72	0.27	2.26	1.09	0.50	12.10	31.20	7.80	1.68	158	15.70
C. mucunoides	44.00	3.52	0.30	2.10	0.83	0.20	16.60	26.70	9.00	1.30	147	12.50
C. spectabilis	53.40	3.06	0.30	1.67	0.86	0.55	11.65	38.47	6.77	0.99	190	17.45
C. cajan	40.44	3.12	0.26	1.32	0.48	0.18	19.92	26.65	10.77	1.39	165	12.96
D. lablab	43.29	3.26	0.42	2.19	0.92	0.32	18.10	26.65	6.75	1.62	143	13.28
S. guyanensis	43.52	3.20	0.26	1.72	1.17	0.25	12.90	29.80	4.80	1.72	168	13.60
S. deeringianum	45.51	3.70	0.26	1.97	0.82	0.20	16.90	31.70	8.60	2.04	174	12.30
Spontaneous	65.55	2.38	0.26	3.03	1.04	0.46	20.92	29.54	9.27	1.31	70.45	27.54

HM: hemicellulose; CL: cellulose; LG: lignin, and PP: total soluble polyphenols; C/P: carbon/phosphorus ratio; C/N: carbon/nitrogen ratio.

plants in this case). The $\delta^{15}N$ is defined as: $\delta^{15}N$ (‰) = $[(R_{sample} - R_{standard})/R_{standard}] \times 1000$, in which R_{sample} is the isotopic ratio $^{15}N/^{14}N$ of the sample, and $R_{standard}$ is the isotopic ratio $^{15}N/^{14}N$ of the standard. For N, the international standard is atmospheric N (0.3663 %). To estimate the contribution of the BNF, the equation cited by Boddey et al. (2000) is used: $[(\delta^{15}N_{control\ plant} - \delta^{15}N_{test\ plant})/(\delta^{15}N_{control\ plant} - \beta)]$, in which $\delta^{15}N_{control\ plant}$ is the value of the $\delta^{15}N$ of the N taken up from the soil, obtained in leaves of the spontaneous plants used as the non-fixing reference; $\delta^{15}N_{test\ plant}$ is the value of the $\delta^{15}N$ of the N_2 fixing plant; and β is the value of the isotopic discrimination of ^{15}N made by the plants during the BNF process, using the value of -0.9, which was estimated for *Cajanus cajan* (Boddey et al., 2000). The amount of N derived from N_2 fixation was obtained by the product of the BNF% and the total N accumulated in the aerial part of the legume (Boddey et al., 2000).

The contribution of N from N-BNF to the N levels in coffee leaves was determined according to Boddey et al. (2000), just as was done for legumes. The natural abundance technique allows quantification of the N-BNF present in coffee leaves.

The data were subjected to analysis of variance using the F-test and comparison of the means by the Scott-Knott test, with a probability of 0.05. The SAEG 5.0 statistical program was used to perform these analyses (Funarbe, 1993).

RESULTS

Analysis of variance showed a significant effect on all the variables analyzed ($p<0.05$). The production of fresh and dry matter from the legumes and spontaneous plants is shown in figure 1. The perennial cycle legumes *A. pintoi*, *C. mucunoides*, and *S. guianensis* obtained the lowest yields of fresh matter (5.07 to 8.06 Mg ha^{-1}) and dry matter (1.27 to 2.07 Mg ha^{-1}). The annual cycle legumes *C. spectabilis*, *D. lablab*, *S. deeringianum*, and *C. cajan* obtained the highest yields for fresh matter, with 14.40, 11.21, 11.17, and 11.02 Mg ha^{-1}, respectively. The highest yields for dry matter were obtained for *C. spectabilis* (3.04 Mg ha^{-1}) and *C. cajan* (3.01 Mg ha^{-1}).

The natural abundance of ^{13}C ($d^{13}C$) and ^{15}N ($\delta^{15}N$) differed among treatments ($p<0.05$). The $d^{13}C$ is indicative of the variability of the active vegetation used as green manure with the coffee plants. The ^{13}C values ranged from -30.94 ‰ for *S. muyanensis* to -16.97 ‰ for spontaneous plants (Figure 2). For the ^{15}N values, the range was 8.66 ‰ for *C. mucunoides to* 16.80 ‰ for spontaneous plants (Figure 2), showing differences in BNF among species.

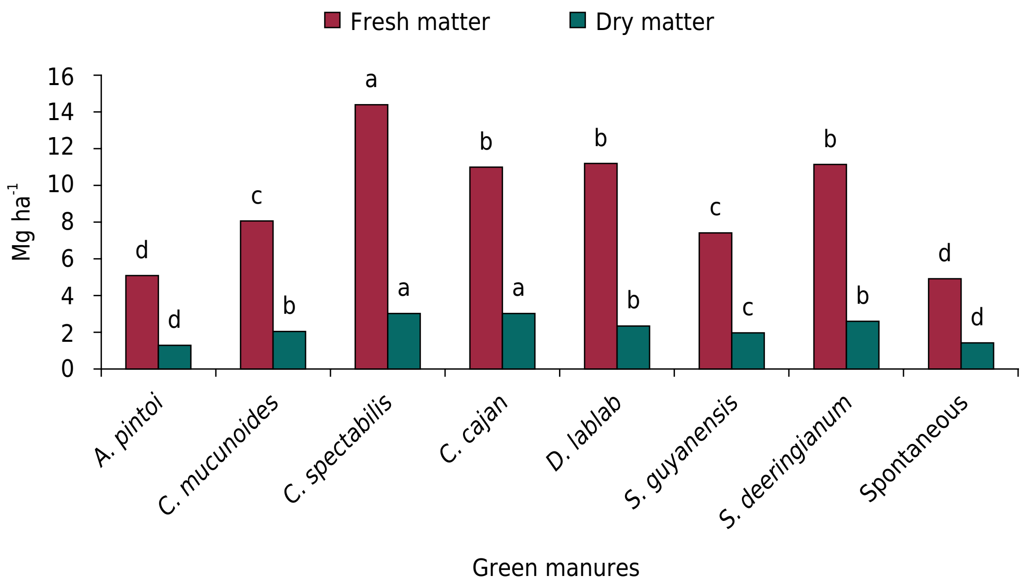

Figure 1. Fresh and dry matter of the legumes and spontaneous plants intercropped with coffee in the Zona da Mata region, state of Minas Gerais, Brazil. Means followed by the same letter for each variable do not differ at the 5 % level of probability by the Scott-Knott test.

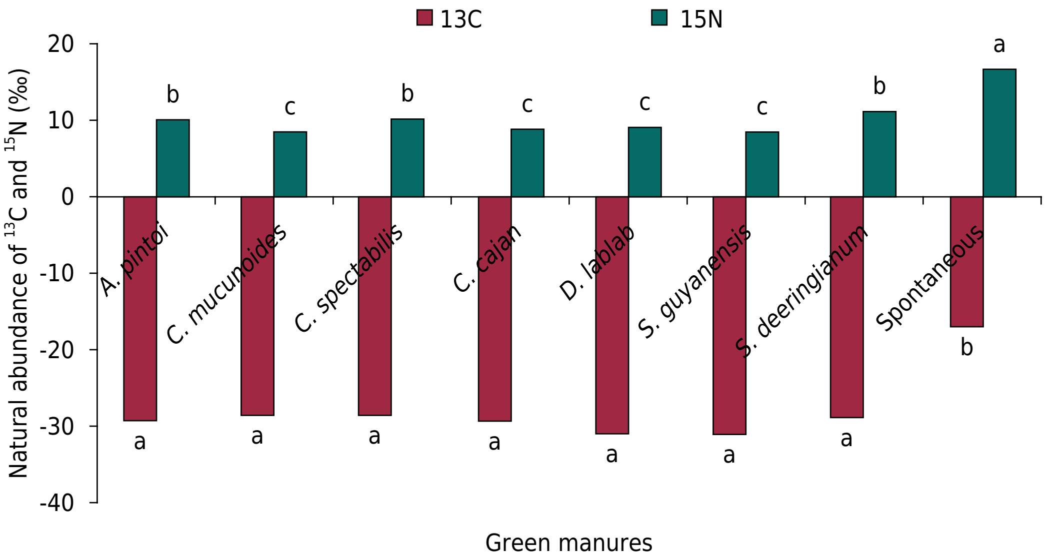

Figure 2. Natural abundance of ^{13}C ($\delta^{13}C$) and ^{15}N ($\delta^{15}N$) for dry matter of the legumes and spontaneous plants intercropped with coffee in the Zona da Mata region, state of Minas Gerais, Brazil. Means followed by the same letter do not differ at the 5 % level of probability by the Scott-Knott test.

Because of differences in the $\delta^{15}N$, the BNF rate ranged from 30.8 % for *S. deeringianum* to 46.1 % for *C. mucunoides*, which indicates differences in the fixation potential of the legumes (Table 3). For *C. spectabilis*, the total accumulation of N was 93.42 kg ha^{-1}; and 34.10 kg ha^{-1} was derived from BNF (Table 3). Of the total N accumulated by *C. cajan* (100.05 kg ha^{-1}), 44.42 kg ha^{1} was derived from BNF, thus indicating its use as a strategy for supplying N to the soil and supplementing N fertilization.

The amounts of total N obtained from the leaves of the coffee plants intercropped with the different green manures ranged from 24.3 g kg^{-1} for the spontaneous plants to 40.3 g kg^{-1} for *C. spectabilis* (Table 4). Among the green manures, *C. cajan* was the species that made the greatest transfer of N-BNF to the coffee leaves (55.8 %), which corresponds to 21.8 g kg^{-1}. A share of 19.7 g kg^{-1} of N in coffee leaves was from the BNF of *C. spectabilis*.

Table 3. N_2 fixation rate, total accumulation of nitrogen, nitrogen derived from biological fixation, and nitrogen derived from the soil in the green manure crop intercropped with coffee in the Zona da Mata region, state of Minas Gerais, Brazil

Green manure	N_2 fixation rate	Accumulation of N	N derived from fixation	N derived from soil
	%		kg ha^{-1}	
A. pintoi	37.7 b	36.73 d	13.84 d	22.89 d
C. mucunoides	46.1 a	63.28 c	29.17 c	34.11 c
C. spectabilis	36.5 b	93.42 a	34.10 b	59.32 a
C. cajan	44.4 a	100.05 a	44.42 a	55.63 a
D. lablab	42.9 a	77.85 b	33.40 b	44.45 b
S. guyanensis	45.9 a	60.09 c	27.58 c	32.51 c
S. deeringianum	30.8 c	83.63 b	25.76 c	57.87 a
Spontaneous plants		41.32 d	-	-

Means followed by the same letter in the column do not differ at the 5 % level of probability by the Scott-Knott test. The coefficient of variation of all data was about 15 to 25 %.

Table 4. Leaf nitrogen levels of the coffee plants, natural abundance of ^{15}N ($\delta^{15}N$) in coffee leaves, the contribution of nitrogen from N-BNF to the nitrogen levels in coffee leaves, and the transfer of nitrogen from the legumes and from the soil to the leaves of the coffee plants in the Zona da Mata region, state of Minas Gerais, Brazil

Green manure	Leaf N	$\delta^{15}N$	N-BNF	Transfer of N from legumes	Transfer of N from soil
	g kg^{-1}	‰	%	g kg^{-1}	
A. pintoi	29.2 b	9.58 b	39.6 c	11.6 c	17.6 c
C. mucunoides	32.0 b	8.23 c	48.1 b	15.4c	16.6 c
C. spectabilis	40.3 a	8.13 c	48.8 b	19.7 b	20.6 b
C. cajan	39.1 a	7.02 c	55.8 a	21.8 a	17.3 c
D. lablab	36.8 a	9.78 b	38.4 c	14.1 c	22.7 b
S. guyanensis	29.6 b	9.72 b	38.8 c	11.5 c	18.1 c
S. deeringianum	37.4 a	10.12 b	36.2 c	13.5 c	23.9 a
Spontaneous plants	24.3 c	15.87 a	-	-	-

Means followed by the same letter in the column do not differ at the 5 % level of probability by the Scott-Knott test. The coefficient of variation of all data was about 15 to 25 %.

DISCUSSION

The difference between the fresh and dry matter of the legumes is due to the specific nature of the development cycle of each species. Another factor that affects the production potential of each legume is the ability to take up nutrients (Ribaski et al., 2001). According to these authors, in conditions of low soil fertility, such as in the soils studied, the difference in yield among the species may be due to the greater ability of one of them to take up nutrients that are less available to the plants. Just as in this present study, Matos et al. (2008) diagnosed, among the legumes studied, lower dry matter production for *A. pintoi*. Teodoro et al. (2011) reported dry matter of 5.65, 5.45, and 2.62 Mg ha^{-1} for *D. lablab, C. spectabilis*, and *C. cajan*, respectively. The productive potential of legumes in a short period of time, as occurred for *C. spectabilis* and *C. cajan*, reveals that these species have potential for cultivation in the Zona da Mata region of Minas Gerais (Perin et al., 2004).

The $d^{13}C$ values can be differentiated among the two groups of plants – C3 and C4 – since the C fractionation process occurs in distinct photosynthetic pathways (Pereira, 2007). The data obtained in this study reinforce the endogeneity of the plants present during intercropping with coffee, excluding the possibility of other plants being present together with the species of interest, given that mechanical control was undertaken to remove the different spontaneous species that germinated among the legumes.

Unlike what happens with C, for N there is no fractionation in the plant and there is also greater reuse of it by the crop (Pereira, 2007). Thus, the $\delta^{15}N$ in the plant resembles the $\delta^{15}N$ of its source, and so variation in the composition of the N source may reveal plants with different isotopic signatures (which is the case for these legumes), given that each species has different abilities in BNF, which results in differences in the $\delta^{15}N$ (Figure 2). Since soil contains more ^{15}N than the atmosphere, the plants that fix the atmospheric N are depleted in terms of ^{15}N in relation to the non-fixing plants (Gannes et al., 1998), which explains the higher $\delta^{15}N$ in the spontaneous plants.

The differences in BNF rates are related both to the ability of each legume to take up the mineral N of the soil and the efficiency of BNF via the rhizobia population native to the soil, given that inoculation was not performed (Perin et al., 2004). The BNF contribution to the crotalaria (*C. juncea*) inoculated with the rhizobia bacteria of the BR 2001 strain was 57 % (Perin et al., 2004). Paulino et al. (2009) reported BNF of 64.5 % for *C. juncea* inoculated with the rhizobia bacteria of the BR 2003 strain (SEMIA 6156). These data demonstrate that legume seeds should be inoculated, since this leads to increases in BNF and greater accumulation of N in the legumes.

Improvement in the balance of N through the introduction of legumes in green manure crops is particularly important in tropical soils as they are initially poor in this nutrient, a factor that limits coffee production. Therefore, introduction of legumes results in use of less N fertilizer, which subsequently ensures greater sustainability of agroecosystems (Perin et al., 2004).

Considering the critical range of N sufficiency (29.0 to 32.0 g kg^{-1}) in the leaves of Arabica coffee plants (Guimarães et al., 1999; Prezotti et al., 2007), it appears that only in the coffee intercropped with spontaneous plants are the coffee leaf N levels below the critical range. Among the legumes, *C. cajan* (leaf level of 39.1 g kg^{-1}) was the species that most transferred the N-BNF (55.8 %). Paulino et al. (2009) reported that crotalaria transferred 22.5 % of the N-BNF to the soursop trees. The BNF of the tropical leguminous kudzu (*Pueraria phaseoloides*), pinto peanuts (*Arachis pintoi*), and siratro (*Macroptilium atropurpureum*) contributed 33.7, 40.5, and 24.2 %, respectively, of the N found in the tissue of banana leaves (Espindola et al., 2006).

The results of this study indicate that the use of legumes intercropped with coffee plants is important in supplying N, as well as in transferring N derived from BNF for nutrition of the coffee plants.

CONCLUSIONS

Under the experimental conditions tested, *Calopogonium mucunoides*, *Stylosanthes guianensis*, *Cajanus cajan*, and *Dolichos lablab* have greater capacity for fixing N than the legumes *Arachis pintoi*, *Crotalaria spectabilis*, and *Stizolobium deeringianum*.

Cajanus cajan is the legume that contributes the greatest amount of N to the soil via biological N fixation.

Cajanus cajan, *C. spectabilis*, and *C. mucunoides* are legumes that have high potential for transferring N to coffee plants, and are recommended for intercropping with the coffee crop.

Legumes intercropped with coffee are an excellent alternative to N fertilization of coffee grown by family farmers with crops in the mountainous region of the Atlantic Forest Biome.

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