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The ^{15}N isotope to evaluate fertilizer nitrogen absorption efficiency by the coffee plant

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ABSTRACT

The use of the ^{15}N label for agronomic research involving nitrogen (N) cycling and the fate of fertilizer-N is well established, however, in the case of long term experimentation with perennial crops like citrus, coffee and rubber tree, there are still shortcomings mainly due to large plant size, sampling procedures, detection levels and interferences on the system. This report tries to contribute methodologically to the design and development of ^{15}N labeled fertilizer experiments, using as an example a coffee crop fertilized with ^{15}N labeled ammonium sulfate, which was followed for two years. The N of the plant derived from the fertilizer was studied in the different parts of the coffee plant in order to evaluate its distribution within the plant and the agronomic efficiency of the fertilizer application practice. An enrichment of the fertilizer-N of the order of 2% ^{15}N abundance was sufficient to study N absorption rates and to establish fertilizer-N balances after one and two years of coffee cropping. The main source of errors in the estimated values lies in the inherent variability among field replicates and not in the measurements of N contents and ^{15}N enrichments of plant material by mass-spectrometry.

Key words: experimental design, replicate variability, stable isotope methodology, perennial crop.

INTRODUCTION

The study of soil-plant relationships in agricultural crops through the use of radioactive or stable isotopes as tracers is well established and successfully achieved in a variety of situations (Reichardt and Bacchi 2004). In the case of experimentation over long periods of time the employment of radioactive isotopes becomes limited in many cases due to the inexistence of a specific isotope of a sufficiently long half life that would be compatible with the experimental period, allowing its detection up to the end of the evaluations. In these cases, when a specific and suitable stable isotope is available for the study, its use is more advantageous in relation to the radioisotopes.

For studies on nitrogen (N) cycling involving both environmental and agronomic aspects, the isotope ^{15}N has been extensively used as a label of natural changes of the $^{15}N/^{14}N$ ratio through $\delta‰$ values or as a label of imposed changes of ^{15}N abundances, employing nitrogenous materials enriched to levels much above the natural ^{15}N abundance. Hardarson (1990) thoroughly explained the methodological aspects of the use of the ^{15}N tracer in agronomic research, indicating its viability and establishing procedures for its correct use.

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In the agronomic literature the number of studies that employ ^{15}N as a tracer is high for annual crops, crop sequences and management practices, either planted in the field or cultivated in controlled environments. For perennial crops, however, such studies are published in a much lower volume, mainly due to the larger stature of the plants and longer life cycle, which lead to specific problems related to the ^{15}N tracer use. As examples we cite the reports of Wallace et al. (1954), Legaz et al. (1982), Feigenbaum et al. (1987), Legaz et al. (1995), Boaretto et al. (1999a, b), Lea-Cox et al. (2001), Lima Filho and Malavolta (2003), and Fenilli et al. (2004) who worked with the genus Citrus, and Bustamante et al. (1997), Snoeck et al. (1998) and Snoeck and Domenach (1999) with the genus Coffea.

The main difficulties that arise with the use of the ^{15}N tracer in perennial crops are related to the large plant size, which requires the use of great quantities of the label, and leads to a high experimental cost in terms of materials and isotope detection through mass-spectrometry. Representative sampling becomes more difficult due to the sizes of the samples to be collected, in general being whole plans of large size and age, which implies in representative sub-sampling. Based on an experiment carried out on a coffee crop fertilized with ^{15}N labeled ammonium sulfate, with the aim of studying the absorption rates of the fertilizer-N by the coffee plant (Fenilli et al. 2007) and the fate of the fertilizer-N in the soil-plant-atmosphere system (T.A.B. Fenilli et al., unpublished data), this study discusses aspects of enrichment levels, analytical isotopic errors in comparison to the inherent field variability, and ^{15}N enrichment variability among the different plant parts.

MATERIALS AND METHODS

Study Area and Experimental Design

Field studies were conducted from 2003 to 2005, at the Agricultural Campus of the University of São Paulo, Research Station of Piracicaba, SP, Brazil (22°42′S, 47°38′W, 580 m above sea level) on a Typic Rhodudalfs according to US Soil Taxonomy, named Nitossolo Vermelho Eutroférrico according to Brazilian classification system (Embrapa 2006). Details on the study area and its climate are described elsewhere (Fenilli et al. 2007, Silva et al. 2006).

Coffee seedlings (*Coffea arabica* L.) of the variety "Catuaí vermelho IAC-144" were planted in rows along contour lines in May 2001, with a row spacing of 1.75 m and 0.75 m between plants, with a population of 7.620 plants per ha. Coffee is a perennial crop which starts producing beans in the third year. In Brazil the crop cycle begins with flowering at the end of the cold and dry season, as a consequence of the first significant rain, which occurs in the Piracicaba region during August-September. Fruit setting, grain filling and maturation take 9-10 months so that harvest is made between May and June. Therefore this fertilizer trial started on September 1, 2003 when plants started blooming and were 1.2 m tall, and continued during two years, until August 30, 2005. Time was measured as days after Beginning (DAB), 0 DAB corresponding to Sept. 1, 2003, 8:00 a.m.

To carry out the experiment five plots of about 120 plants each were randomly selected in an area of about 0.2 ha of the established coffee crop, to receive N fertilizer at the rates of: 280 kg ha^{-1} of N in 2003/2004 and 350 kg ha^{-1} of N in 2004/2005, supplied as ammonium sulfate, split into four applications: September 1 and 60, 45 and 45 days after, for the two years. Within each of these five plots (replicates), sub-plots of sequences of three plants of one row were chosen for the N labeled fertilizer study. The ammonium sulfate was enriched at 2.072 ± 0.001 ^{15}N atom percent for both years' applications. The labeled fertilizer was carefully and homogeneously broadcast below plant canopy, over the dead leaf mulch, according to the most commonly adopted practice.

Plant Sampling and Analysis

For total shoot dry matter one whole plant per replicate was harvested outside the isotope row at each sampling time. The chosen plant was very similar to the central one of the three labeled plants, so that it could be assumed to represent the labeled one in terms of growth and yield. They were dissected into parts called compartments (C) as follows: C_1 – central stem or orthotropic branch (OB); C_2 – productive plagiotropic branches (PB); C_3 – leaves of productive branches (LPB); C_4 – Vegetative plagiotropic branches (VB); C_5 – leaves of vegetative branches (LVB); C_6 – fruits (beans) (F). These compartments of

each replicate were separated in the laboratory, then oven dried at 65°C and weighed.

Since the central plant of the three labeled plants could not be sacrificed, it was only used for N total and N abundance evaluations collecting one full branch (out of more than 50 branches at the beginning) at each sampling time. One mature branch has samples of compartments C_2 to C_6 and to represent the orthotropic branch C_1, that obviously could not be harvested, we took the first centimeter of C_2 that is in close connection with C_1. This first cm is hardwooden and was assumed to represent the central stem. The sampling of only one full branch per replicate was adopted to minimize interference on the growth and development of the labeled plant. At the end of the experiment plants had already more than 100 branches, so that we assumed that the harvest of nine branches (total number of samplings) for analysis during the two years did not affect significantly plant growth and development. These samples were also oven dried at 65°C and finely ground. Representative sub-samples of 5 μg were used for total N and ^{15}N abundance evaluations by mass spectrometry in an automated continuous flow Mass Spectrometer, Model ANCA-SL (Europa Scientific) as described by Mulvaney (1993) and Barrie and Prosser (1996).

Calculations

Based on data of dry matter (DM, g plant^{-1}), total nitrogen concentration (C_N, %), and ^{15}N enrichment (A_N, atom % in excess of 0.366) for each of the above described compartments, it was possible to calculate the accumulated nitrogen (N_{acc}, g plant^{-1}) in each compartment and the fraction of this nitrogen that is derived from the fertilizer (Ndff, %) (Hardarson 1990):

$$N_{acc} = \frac{DM \cdot C_N}{100} \quad (1)$$

and

$$Ndff = \frac{A_N \text{ of the compartment}}{A_N \text{ of the fertilizer}} \times 100 \quad (2)$$

and also the quantity of nitrogen in each compartment that is derived from the fertilizer (QNdff, g plant^{-1}):

$$QNdff = \frac{N_{acc} \cdot Ndff}{100} \quad (3)$$

Since C_N and A_N vary considerably among the six compartments, their weighted average (WA) was calculated according to:

$$WA = \frac{\sum_{i=1}^{6} [C_N \times mMS]}{\sum_{i=1}^{6} mMS} \quad (4)$$

in which C_N is exchanged by A_N in order to obtain the WA for ^{15}N enrichment. These averages are also compared with C_N and A_N data of each compartment in order to find out which of them would better represent the whole plant. This would reduce the number of samples for analysis and the experimental cost in future experiments using the ^{15}N label in coffee.

Data were statistically analyzed using the descriptive concepts of the mean of n (five) replicates and its standard error $s_m = sd/\sqrt{n}$, where sd is the standard deviation. Relations between variables were quantified using linear regression.

RESULTS AND DISCUSSION

The evolution of plant shoot DM (Table I) during the 636 days of this study, obtained by harvesting at each date five whole plants (one per replicate) shows more than half of the standard errors s_m above 10% of the respective means. Although the plants were chosen based on a similarity criterium, the DM of each compartment varied considerably, indicating a large number n of replicates being necessary. However, harvesting more than five large perennial plants at each sampling time would determine a great impact on the plant stand, interfering in the growth and development of the crop as a whole. The total shoot DM, however, presented a smaller variability, always below 10%.

The total-N concentration C_N (Table II) varied strongly among compartments and time, as a function of the application of the readily available fertilizer-N and depending on the N redistribution within the plant, including roots. The standard errors shown in brackets include the measurement error of C_N performed during the mass-spectrometry procedure, and the variability of the five replicates. The evaluation of C_N by the mass-spectrometer involves the calibration with standard C_N samples, which are included in the measurement se-

TABLE I
Means and standard errors (s_m) of dry matter yield of the different compartments of the coffee plant shoot as a function of time (DAB = days after beginning, starting Sept 1, 2003).

DAB	Compartment						
	OB	PB	LPB	VB	LVB	F	Total
	g plant^{-1}						
63	304.9 (± 40.4)	64.5 (± 13.0)	63.1 (± 17.8)	55.8 (± 10.3)	201.9 (± 16.2)	3.1 (± 0.8)	693.4 (± 69.2)
126	371.1 (± 33.7)	109.2 (± 13.3)	153.0 (± 33.0)	64.8 (± 10.5)	369.3 (± 47.1)	39.3 (± 15.1)	1106.7 (± 104.1)
182	413.7 (± 41.1)	180.1 (± 14.9)	306.7 (± 46.2)	98.4 (± 9.2)	509.0 (± 50.3)	153.2 (± 41.2)	1661.2 (± 125.5)
243	578.0 (± 42.6)	198.7 (± 9.4)	222.0 (± 8.6)	193.0 (± 24.4)	849.4 (± 110.5)	182.4 (± 56.4)	2223.5 (± 161.7)
366	741.5 (± 73.8)	220.3 (± 14.1)	43.04 (± 8.6)	99.6 (± 9.5)	249.9 (± 26.2)	40.2 (± 6.3)	1394.4 (± 90.3)
430	796.2 (± 63.6)	427.4 (± 51.5)	84.0 (± 13.9)	71.7 (± 5.6)	308.3 (± 24.6)	80.0 (± 12.7)	1767.5 (± 109.7)
491	763.5 (± 54.7)	308.4 (± 8.4)	38.0 (± 4.7)	65.9 (± 3.3)	408.3 (± 20.3)	577.7 (± 45.2)	2161.9 (± 103.5)
548	917.8 (± 115.9)	328.7 (± 42.6)	29.3 (± 8.2)	112.6 (± 9.3)	729.2 (± 73.5)	895.5 (± 120.8)	3013.0 (± 225.2)
636	1080.9 (± 73.5)	339.6 (±1 3.8)	21.5 (± 5.5)	146.6 (± 16.3)	756.3 (± 24.2)	1598.5 (± 258.3)	3943.3 (± 243.8)

OB – orthotropic branch; PB – productive plagiotropic branches; LPB – leaves of productive branches; VB – vegetative plagiotropic branches; LVB – leaves of vegetative branches; F – fruits.

quence after every 10 samples. This standard error is in the order of 1%. The s_m presented in Table II are in the order of 4% of the mean, some however above this value, the highest of them corresponding to vegetative branches at 491 DAB, with a value of 15.9% of the mean. In view of the small mass-spectrometry error, these high standard errors are certainly mostly due to the inherent variability among replicates of an agronomic field experiment.

The ^{15}N enrichment A_N (Table III) also varied among compartments and in time, depending on how much ^{15}N fertilizer was applied before each sampling time. Values increase from 63 to 243 DAB with a reduction at 366 DAB due to leaf fall, translocation of N among compartments including the root system, and fruit export at harvest (Fenilli et al. 2007). The same pattern was observed for the second year (366 to 636 DAB). The s_m also include the measurement error by mass-spectrometry (of the order of 0.1%) and the agronomic variability of the five replicates. This last variability depends on the homogeneity of the fertilizer broadcasting procedure, of the flow of the fertilizer into the soil, of the root distribution and its activity, and on the translocation to the shoot. The s_m values presented in Table III are of the order of 6% of the mean, with highest values at 430 DAB (10.2%) for LPB, and at 491 DAB (10.1%) for VB, also certainly due to the variability among field replicates. Evaluations of An in fruit (F), made in 2006 (1,001 DAB) and in 2007 (1,366 DAB), were 0.250 and 0.236 atom % in excess, respectively, showing that the label was still at easy detectable levels two years after the end of the experiment.

The quantities of N derived from the fertilizer QNdff obtained through equation 3, are presented in Table IV. With a small number of exceptions, standard errors varied between 10 and 25%, the highest corresponding to

TABLE II
Means and standard errors (s_m) of total-N concentration (C_N) as a function of time (DAB = days after beginning, starting Sept 1, 2003) for the different aerial compartments of the coffee plant, measured during mass-spectrometry.

DAB	Compartment					
	OB	PB	LPB	VB	LVB	F
	%					
63	1.45 (± 0.07)	1.71 (± 0.14)	3.50 (± 0.21)	2.48 (± 0.11)	4.26 (± 0.11)	4.70 (± 0.14)
126	1.51 (± 0.12)	1.91 (± 0.15)	3.87 (± 0.16)	2.51 (± 0.09)	4.05 (± 0.15)	2.93 (± 0.39)
182	1.53 (± 0.09)	2.04 (± 0.12)	4.01 (± 0.13)	2.36 (± 0.12)	3.96 (± 0.15)	3.18 (± 0.16)
243	1.49 (± 0.11)	1.77 (± 0.13)	3.23 (± 0.11)	2.07 (± 0.09)	3.54 (± 0.13)	2.74 (± 0.19)
366	0.94 (± 0.08)	1.42 (± 0.16)	2.31 (± 0.22)	2.02 (± 0.22)	2.58 (± 0.27)	3.05 (± 0.28)
430	1.04 (± 0.07)	1.65 (± 0.10)	3.01 (± 0.06)	2.15 (± 0.07)	3.68 (± 0.06)	4.05 (± 0.08)
491	0.98 (± 0.08)	1.28 (± 0.11)	2.78 (± 0.05)	1.68 (± 0.26)	3.21 (± 0.16)	2.69 (± 0.20)
548	0.96 (± 0.04)	1.19 (± 0.05)	2.56 (± 0.07)	1.88 (± 0.10)	3.05 (± 0.06)	2.00 (± 0.09)
636	1.07 (± 0.12)	1.53 (± 0.14)	2.33 (± 0.07)	1.74 (± 0.13)	2.72 (± 0.11)	2.31 (± 0.15)

OB – orthotropic branch; PB – productive plagiotropic branches; LPB – leaves of productive branches; VB – vegetative plagiotropic branches; LVB – leaves of vegetative branches; F – fruits.

TABLE III
Means and standard errors (s_m) of ^{15}N enrichment as a function of time (DAB = days after beginning, starting Sept 1, 2003) for the different aerial compartments of the coffee plant, measured by mass spectrometry.

DAB	Compartment					
	OB	PB	LPB	VB	LVB	F
	^{15}N atom % in excess to 0.366					
63	0.187 (± 0.01)	0.229 (± 0.01)	0.392 (± 0.02)	0.403 (± 0.02)	0.505 (± 0.02)	0.387 (± 0.02)
126	0.509 (± 0.03)	0.523 (± 0.03)	0.734 (± 0.06)	0.741 (± 0.04)	0.844 (± 0.07)	0.774 (± 0.05)
182	0.721 (± 0.04)	0.883 (± 0.04)	0.953 (± 0.03)	0.939 (± 0.05)	0.928 (± 0.05)	0.878 (± 0.06)
243	0.843 (± 0.06)	1.029 (± 0.05)	1.041 (± 0.06)	1.017 (± 0.07)	1.027 (± 0.05)	0.963 (± 0.06)
366	0.576 (± 0.03)	0,647 (± 0.04)	0.766 (± 0.06)	0.621 (± 0.05)	0.651 (± 0.06)	0.600 (± 0.04)
430	0.682 (± 0.05)	0.715 (± 0.06)	0.785 (± 0.08)	0.781 (± 0.06)	0.801 (± 0.07)	0.744 (± 0.07)
491	0.811 (± 0.05)	0.815 (± 0.05)	0.741 (± 0.03)	0.787 (± 0.08)	0.819 (± 0.06)	0.831 (± 0.08)
548	0.804 (± 0.04)	0.843 (± 0.04)	0.866 (± 0.06)	0.954 (± 0.05)	0.946 (± 0.05)	0.932 (± 0.06)
636	0.735 (± 0.04)	0.781 (± 0.04)	0.847 (± 0.05)	0.814 (± 0.05)	0.866 (± 0.05)	0.887 (± 0.04)

OB – orthotropic branch; PB – productive plagiotropic branches; LPB – leaves of productive branches; VB – vegetative plagiotropic branches; LVB – leaves of vegetative branches; F – fruits.

fruit at 126 DAB (45.2%). These errors are large, however acceptable for several types of agronomic experimentation (Pimentel Gomes 1970). The high values correspond to early stages of the experiment, when the fertilizer was still penetrating the soil and plants had not absorbed much of it. Another reason for these errors are the small values of DM of the individual compartments. Values of $QNdff_1$ shown in the first column of Table V

represent the whole plant and are the sums of the QNdff of the compartments presented in Table IV, and there it can be seen the standard errors in the order of 10% of the respective means, are small with the highest value for 243 DAB (15.6%).

In order to verify which compartment best represents the whole plant in terms of C_N and A_N, linear regressions were made between WA and C_N or A_N, for each compartment, using data of the nine samplings made during the two years of the experiment. These regressions are presented in Figure 1, all with significant values of R^2, with exception to fruit for C_N. From the theoretical point of view, the best regressions would be those with slope closest to the 1, intercept closest to 0 and with high R^2. For C_N the closest would be for LPB and LVB, and for A_N PB, LPB, LVB, and F. However, from the practical point of view, LVB are always present, easy to be sampled with minimal interference on the growth and development of the plant, and could therefore be chosen as the compartment that best represents the whole plant, for both C_N and A_N simultaneously. Their regressions have also high and significant R^2 and are:

$$WA_C = 0.7626C_N(LVB) - 0.4278;\ R^2 = 0.7553 \quad (5)$$

$$WA_A = 1.0867A_N(LVB) - 0.1384;\ R^2 = 0.9079 \quad (6)$$

In Table V we compare QNdff data calculated in three ways: $QNdff_1$ as already described; $QNdff_2$ using the WA for C_N and A_N; and $QNdff_3$ using regressions (5) and (6) to find out the WA using C_N and A_N for LVB. The statistical analysis shows that there is no difference between the data for all dates, showing that the LPB can be used in this kind of experimentation, significantly reducing sampling procedures and costs.

FINAL CONSIDERATIONS

As discussed above, the precision and accuracy involved in the use of ^{15}N fertilizer in studies of N recovery and balance can be separated in two sources of error: i) those involving the analytic measurement of the nitrogen concentration C_N and the ^{15}N enrichment A_N by mass-spectrometry, and ii) those coming from agronomic, sampling and design problems during the execution of the experiment. The evolution of mass-spectrometry was enormous in the last decades, so that natural variations in ^{15}N abundance can easily and safely be detected, making studies with $\delta‰$ very viable. As already said, for enriched materials the standard errors can be as low as 0.1% for A_N. The same is also valid for total N concentration, measured simultaneously in the mass-spectrometer, also with errors of less than 1%. Therefore, the analytic measurement errors are overwhelmed by the agronomic errors which depend on the way by which the experimental field work is carried out.

In our case, the data presented here belong only to one treatment which used the ^{15}N label, of a larger experiment. The number of five replicates used here is the result of a randomized block design including three treatments: T_o (no N fertilizer); T_1 (half N rate); and T_2 (full N rate as described in Materials and Methods). In order to have the residual number of degrees of freedom greater than 10 (Pimentel Gomes 1970) the minimum number of replicates should be five. This number of replicates was also used in the labeled experiment, carried out only on T_2 in order to reduce experimental costs. The variability among these replicates included management practices, plant growth and development variation, homogeneity of label application, sampling, among others. As a result, data on QNdff presented in Table V exhibited standard errors of the order of 15% and, if these values should be reduced, say to half, one could estimate the number (n) of replicates necessary using the approach presented by Warrick and Nielsen (1980):

$$n = [x(\alpha)]^2 \cdot \frac{(sd)^2}{d^2} \quad (7)$$

in which $x(\alpha)$ is the normalized deviation which can be found tabulated (student's t), which is 1.96 at the 0.05 confidence value for infinite degrees of freedom; and d is the desired deviation from the mean. For our average standard deviation sd = 2.5, with a desire of having d = 1/2 sd, the result would be n = 15. In most cases such a high number of replicates would be prohibitive. Therefore we conclude that the standard errors presented in Table V were acceptable, and that in this way the enrichment of 2% of the ^{15}N fertilizer was adequate to study the fate of the fertilizer-N in the coffee crop over a period of two years.

TABLE IV

Quantities of N derived from the fertilizer and accumulated in each compartment of the coffee plant (QNdff) as a function of time (DAB = days after beginning, starting Sept 1, 2003).

DAB	Compartment					
	OB	PB	LPB	VB	LVB	F
	g plant^{-1}					
63	0.48 (± 0.06)	0.15 (± 0.03)	0.54 (± 0.18)	0.34 (± 0.09)	2.55 (± 0.24)	0.03 (± 0.01)
126	1.63 (± 0.12)	0.63 (± 0.08)	2.42 (± 0.47)	0.69 (± 0.09)	7.12 (± 0.60)	0.62 (± 0.28)
182	2.62 (± 0.17)	1.86 (± 0.10)	6.66 (± 0.68)	1.25 (± 0.06)	10.80 (± 0.87)	2.43 (± 0.71)
243	4.24 (± 0.54)	2.14 (± 0.27)	4.43 (± 0.48)	2.46 (± 0.55)	18.46 (± 3.25)	2.83 (± 0.81)
366	2.33 (± 0.34)	1.24 (± 0.26)	0.49 (± 0.14)	0.72 (± 0.10)	2.49 (± 0.42)	0.44 (± 0.10)
430	3.27 (± 0.31)	2.80 (± 0.44)	1.22 (± 0.29)	0.71 (± 0.08)	5.28 (± 0.53)	1.46 (± 0.31)
491	3.62 (± 0.52)	1.92 (± 0.26)	0.46 (± 0.06)	0.53 (± 0.11)	6.25 (± 0.56)	7.55 (± 1.05)
548	4.26 (± 0.78)	1.96 (± 0.32)	0.36 (± 0.08)	1.20 (± 0.16)	12.61 (± 1.96)	9.86 (± 1.50)
636	4.97 (± 0.66)	2.36 (± 0.25)	0.25 (± 0.06)	1.22 (± 0.20)	10.55 (± 1.15)	19.47 (± 3.53)

OB – orthotropic branch; PB – productive plagiotropic branches; LPB – leaves of productive branches; VB – vegetative plagiotropic branches; LVB – leaves of vegetative branches; F – fruits.

TABLE V

Different ways of calculating QNdff: $QNdff_1$ = sum of individual compartments; $QNdff_2$ = use of equation (3) with weighted averages of C_N and A_N; $QNdff_3$ = use of equation (3) with data of C_N and A_N from leaves of productive branches. (DAB = days after beginning, starting Sept 1, 2003).

DAB	$QNdff_1$	$QNdff_2$	$QNdff_3$
day	g plant^{-1}		
63	4.09 (± 0.42)	3.28 (± 0.33)	4.77 (± 0.48)
126	13.11 (± 0.78)	12.05 (± 1.13)	13.38 (± 1.26)
182	25.62 (± 0.96)	25.34 (± 1.92)	22.03 (± 1.66)
243	15.97 (± 2.49)	15.09 (± 1.10)	11.35 (± 0.83)
366	7.72 (± 1.10)	7.43 (± 0.48)	7.15 (± 0.46)
430	14.74 (± 1.51)	14.45 (± 0.90)	18.03 (± 1.12)
491	20.32 (± 2.16)	20.27 (± 0.97)	19.28 (± 0.92)
548	30.24 (± 3.30)	30.95 (± 1.62)	32.52 (± 1.82)
636	38.82 (± 3.23)	37.63 (± 2.33)	30.73 (± 1.90)

Statistical analysis of variance indicated no difference between columns at the $P > 0.01$ level.

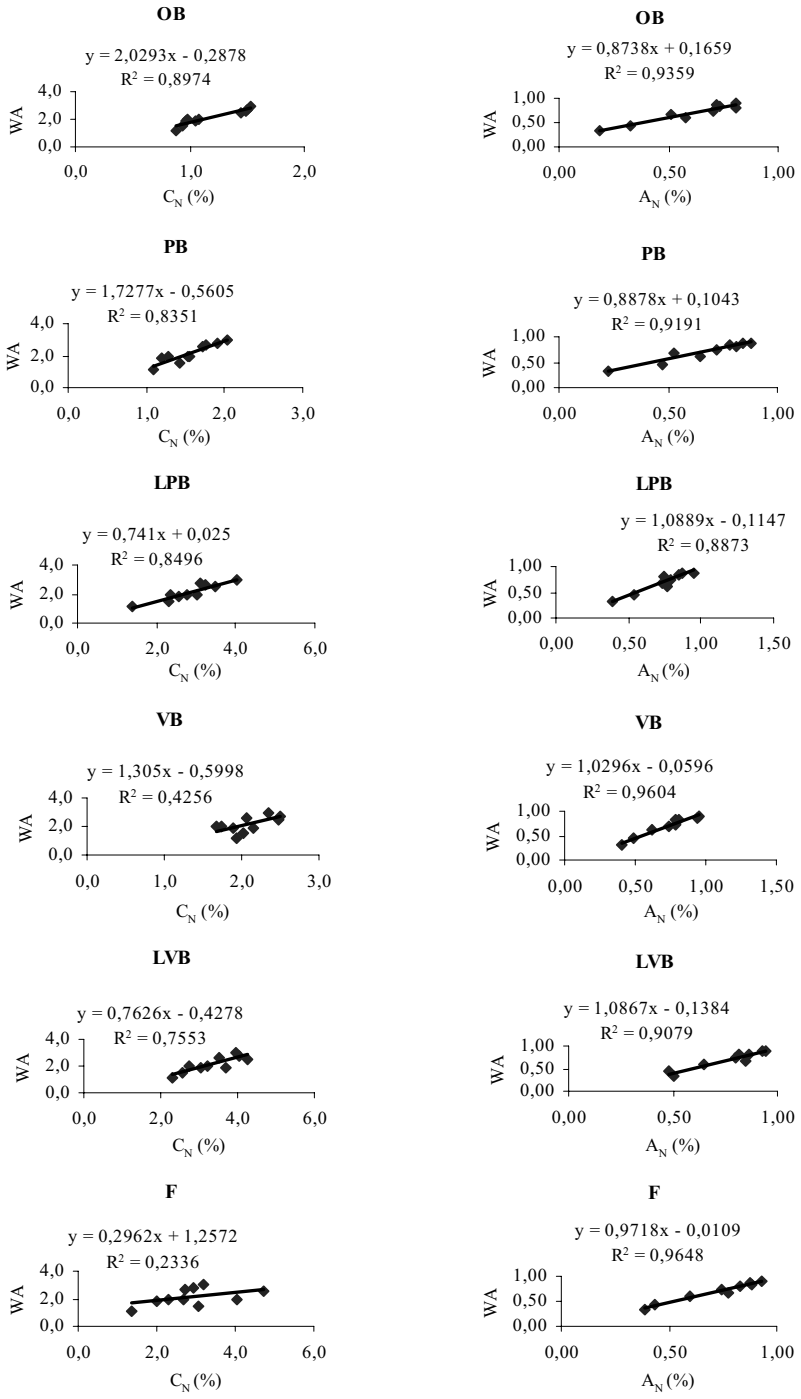

Fig. 1 – Linear regressions between weighted averages (WA of equation 4) and individual values of C_N and A_N for the different compartments, measured at nine sampling times over a two year period (OB = orthotropic branch, PB = plagiotropic branch, LPB = leaf of plagiotropic branch, VB = vegetative branch, LVB = leaf of vegetative branch, and F = fruit).

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RESUMO

O uso do traçador ^{15}N em pesquisas agronômicas que envolvem o ciclo do nitrogênio (N) e o destino do N do fertilizante está bem estabelecido, entretanto, para o caso de experimentação com plantas perenes como citrus, café e seringueira, ainda existem limitações devidas ao porte das plantas, à amostragem, aos níveis de detecção e à interferência no sistema. Este estudo procura contribuir metodologicamente no delineamento experimental e no desenvolvimento desse tipo de experimentação, em condições de campo, fazendo uso, por dois anos, do experimento de uma cultura de café adubada com fertilizante marcado com ^{15}N. O N da planta derivado do fertilizante foi estudado nas diferentes partes da planta de café para determinar sua distribuição dentro dela e a eficiência agronômica da prática de adubação. Um enriquecimento do N do fertilizante da ordem de 2% em abundância de ^{15}N foi suficiente para estudar taxas de absorção de N e estabelecer balanços do N do fertilizante depois de um e dois anos de cultivo. A principal fonte de erros dos valores estimados está na variabilidade agronômica das repetições e não na precisão das medidas de conteúdo de N e de enriquecimento em ^{15}N por espectrometria de massa.

Palavras-chave: delineamento experimental, variabilidade de repetições, metodologia de isótopos estáveis, cultura perene.

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