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Original article

Nutrient Accumulation and Chlorophyll indices in *Coffea canephora* in response to nitrogen rates

Acumulación de nutrientes e índices de clorofila en *Coffea canephora* como respuesta a las dosis de nitrógeno

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ABSTRACT: The objective of this study was to evaluate changes in the concentration and content of macro- and micronutrients in leaves of *Coffea canephora*in response to different nitrogen rates. The experiment was carried out in Colatina-ES, Brazil in conilon coffee plantations of the clonal variety Emcapa 8111, genotype 02. Six nitrogen rates (0, 110, 220, 440, 880 and 1320 kg N ha^{-1}) were evaluated in periodic assessments of growth and yield characteristics. The nutrient concentrations and contents were determined by chemical laboratory analyses. The effects of the N rates on the cumulative levels of N, P, K, Ca and S as well as Fe, Zn, Mn, Cu and B in the coffee leaves depended on the nutrient and the evaluated period. The macronutrient concentrations in the coffee leaves were highest in June. Indirect measurements of the chlorophyll content may be an important tool to diagnose the N status of conilon coffee. For this measurement, the diagnostic leaf is recommended, due to the easy physical access to it on the plant, ensuring a faster field measurement.

Keywords: fertilization, mineral nutrients, irrigation.

RESUMEN: El objetivo de este estudio fue evaluar los cambios en la concentración y contenido de macro y micronutrientes en hojas de *Coffea canephora* en respuesta a diferentes dosis de nitrógeno. El experimento se realizó en Colatina-ES, Brasil, en cafetales conilon de la variedad clonal Emcapa 8111, genotipo 02. Se evaluaron seis dosis de nitrógeno (0, 110, 220, 440, 880 y 1320 kg N ha^{-1}) en evaluaciones periódicas de las características de crecimiento y rendimiento. Las concentraciones y contenidos de nutrientes se determinaron mediante análisis químicos de laboratorio. Los efectos de las dosis de N sobre los niveles acumulados de N, P, K, Ca y S, así como de Fe, Zn, Mn, Cu y B en las hojas de café dependieron del nutriente y del período evaluado. Las concentraciones de macronutrientes en las hojas de café fueron más altas en junio. Las mediciones indirectas del contenido de clorofila pueden ser una herramienta importante para diagnosticar el estado de N del café conilon. Para esta medición se recomienda la hoja de diagnóstico, debido al fácil acceso físico a ella en la planta, asegurando una medición de campo más rápida.

Palabras clave: fertilización, nutrientes minerales, riego.

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INTRODUCTION

Coffee is one of the most appreciated and consumed beverages in the world. Although there are many species of the genus *Coffea*, only two are commercially exploited for consumption: *Coffea arabica* L. and *Coffea canephora* (1). Several countries produce coffee and almost all nations consume it, however 70% of the world supply is produced in only four countries: Brazil, Vietnam, Colombia and Indonesia (1). In this scenario, Brazil is the world's largest coffee producer, where both arabica (*C. arabica*, ca. 65%) as well as conilon/robusta are grown (*Coffea canephora,* ca. 35 %) (2).

Nitrogen (N) is a macronutrient that plays an important role in plant growth and in the metabolic development, in processes such as photosynthesis, nutrient distribution and biomass production (3,4). Nitrogen deficiency can markedly decrease the enzyme activity, chlorophyll content, photosynthesis, respiration rate and plant yield (5) and promote changes in the accumulation of other nutrients (6).

For coffee trees, nitrogen is one of the most universally needed macronutrients (7,8). This makes the balance between a correct N management and a sustainable production a great challenge, since both N excess and deficiency are common problems in coffee production (9,10). Excessive N fertilization, for example, reduces the economic return, decreases yield quality and quantity and increases pollution.

The other macro- and micronutrients are also important for the growth, development and yield of conilon coffee, but the concentrations vary according to the different cultivation conditions (8,11), regions (12), genotypes (13 - 15) phenological stages (11,16) and seasonality (17).

After nitrogen, the second most demanded nutrient with highest cumulative levels in conilon coffee trees is potassium (K), which is exported in large quantities by the coffee trees (7). Potassium is directly related with yield, mainly because of its role in carbohydrate synthesis in the leaves and carbohydrate transport to the fruits and other organs (18). Among the micronutrients with highest cumulative levels are iron (Fe), followed by manganese (Mn), boron (B), zinc (Zn) and copper (Cu) (7,19).

In several studies, a direct relationship was reported between the variation in N concentration and chlorophyll content (20). Thus, chlorophyll measurements can be an auxiliary tool to indirectly diagnose N fertilization requirements.

The relationships between macro- and micronutrient uptake and assimilation with the effects of the amounts of N involved in plant growth and development are complex. Nutrient and chlorophyll concentrations are known to be directly affected by different N availability. The objective of this study was to determine the effects of N rates on macro- and micronutrients and chlorophyll indices of conilon coffee plants.

MATERIAL AND METHODS

Study area

The experiment was carried out on a coffee farm in the district of Colatina, in the northwest of the state of Espírito Santo, Brazil (19° 35' 47" S; 40° 25' 25" W; 83 m asl; mean daily maximum and minimum temperatures 28.2 °C and 12.7 °C, respectively), from 2012 to 2014. The regional climate is tropical, classified as Aw by Köppen's climate classification, with hot humid summers and dry winters (21) and the mean annual rainfall is 1100 mm. During the experimental period, the range of relative humidity in the study area was 49.4% - 92.1%, recorded with a data logger (Log Tag, HAXO-8, China).

The experimental area had a flat topography and the soil was classified as Latossolo Vermelho-Amarelo distrófico (22). The soil chemical properties of the 0 - 40 cm layer were analyzed before the experiment (Table 1).

Experimental design

Three-year-old *Coffea canephora* trees, clonal variety Emcapa 8111, genotype 02, were grown in full sun, at a 3.5 x 1.0 m spacing, pruned and thinned conventionally, to 5-6 branches plant^{-1}, i.e., to a stand density of 13,333 branches ha^{-1}. The management consisted of the commonly applied agronomic practices of fertigation, weeding and chemical insect and pathogen control. Liming and fertilization with other-than-N nutrients were applied as recommended.

The experiment was arranged in a randomized block design with four replications in split plots, where the plots consisted of six nitrogen (N) rates (0, 110, 220, 440, 880 and 1320 kg N ha^{-1} year^{-1}) and the sub-plots of four evaluation periods (E1 - November 2012; E2 - December 2012; E3 - February 2013; E4-June 2013). Each plot consisted of a 7-plant row, of which the five central, but not the two border plants were evaluated. The experiment started in July 2012, when soil sampling, liming, fertilization with other-than-N nutrients and treatments were applied, and ended in June 2014, after coffee harvest. In this way, the evaluations covered the 1st growing season of 2012/2013 and 2nd growing season of 2013/2014.

Assessments of nutrient leaf concentration and content

Leaf samples were collected from the 3rd or 4th pair of leaves counted from the tips of plagiotropic branches of the middle third of the coffee plants, identified as diagnostic leaf (DL) and oldest leaf (OL) of the same branch, on either side of the trees. The samples were inserted in paper bags, dried to constant weight in an oven with forced air circulation at 70 ºC, and weighed on a precision scale to determine dry weight. Subsequently, the samples were ground in a bean mill and analyzed for the macro- (N, P, K, Ca, Mg and S) and micronutrient concentrations (Fe, Zn, Cu, Mn and B), according to the methodology proposed by (23).

Table 1. Chemical and particle size properties of soil samples collected prior to the experiment, from the layers 0-20 and 20-40 cm

Chemical properties	Layer	
	0-20 cm	20-40 cm
pH in water-1: 2.5	5.80	4.60
Organic matter (OM) (dag kg^{-1}) [1]	1.70	1.10
P (mg dm^{-3})[2]	41.00	9.00
K (mg dm^{-3})[2]	64.00	36.00
Ca^{2+} ($cmol_c$ dm^{-3})[3]	1.50	0.80
Mg^{2+} ($cmol_c$ dm^{-3})[3]	0.70	0.30
Exchangeable acidity (Al^{3+}) ($cmol_c$ dm^{-3})[3]	0.00	1.10
Potential acidity (H + Al) ($cmol_c$ dm^{-3})[4]	2.90	4.20
Sum of bases (SB) ($cmol_c$ dm^{-3})	2.40	1.20
Effective CEC (t) ($cmol_c$ dm^{-3})	2.40	2.30
CEC a pH 7.0 (T) ($cmol_c$ dm^{-3})	5.30	5.40
Base saturation (V) (%)	44.90	22.10
Particle-size properties[5]		
Coarse sand (dag kg^{-1})	35	-
Fine sand (dag kg^{-1})	10	-
Silt (dag kg^{-1})	7	-
Clay (dag kg^{-1})	48	-
Physical-water properties		
Field capacity (kg kg^{-1})[6]	0.201	-
Wilting point (kg kg^{-1})[7]	0.126	-
Soil density (kg kg^{-1})[8]	1.1	-

[1]OM=organic carbon or matter x 1.724–Walkley-Black; [2] Mehlich-1 extractor; [3] 1 mol.L^{-1} KCl extractor; [4] 0.5 mol.L^{-1} Calcium acetate extractor pH 7.0; [5] Pipette method" (Embrapa, 1997); [6] Potential -10 kPa; [7] Potential -1500 kPa; [8] Cylinder method

The total leaf nutrient content was calculated as the product of the nutrient concentration of the leaf dry matter (in g kg^{-1}) by the dry weight of the diagnostic leaf (in kg). The oldest leaves were not considered for analyses.

Estimation of chlorophyll concentration

The chlorophyll concentration was measured indirectly with a portable chlorophyll meter (ClorofiLOG CFL1030; FALKER, 2008). The evaluations were carried out in the field in real time, between 7 am and 9 am, on leaves still attached to the plants, with four readings per leaf. The indirect measurements of chlorophyll a, chlorophyll b and total chlorophyll were evaluated. The evaluations were carried out two days after irrigation or rainfall, to ensure a standardized turgor concentration of the leaves.

Statistical analysis

The data were processed by analysis of variance, significant quantitative data were subjected to regression analysis and the models were chosen based on: significance of the regression coefficients (t test at 5% probability); coefficient of determination; and biological logic. The variables were correlated.

RESULTS

The interaction between nitrogen rates and evaluation times was significant ($p \leq 0.05$) for the concentrations and contents of macro- and micronutrients contained in the dry matter of the diagnostic conilon leaf (Tables 2 and 3).

The nitrogen rates influenced the N concentration and content positively. In the first harvest of February 2013, the estimated Critical Levels of the concentrations and contents of macro- and micronutrients for conilon coffee resulted in a yield of 95% of the maximum productivity of conilon coffee when associated with an N rate of 420.7 kg N ha^{-1}. In the 2nd harvest of the other periods (June and October 2013 and February 2014), these levels resulted in the same yield percentage when associated with the application of 543.1 kg N ha^{-1} (Figure 1A; Table 2 and 3). The N concentrations varied between 30.7 g kg^{-1} and 35.9 g kg^{-1} in February (summer) and June 2013 (winter), respectively, followed by decreases in February 2014, in comparison with October 2013 (Table 4).

There was also a positive effect on the concentration and content of phosphorus and potassium, since the increasing N rates raised these indices in February 2013 and 2014. There was a reduction in P and K concentrations in October 2013 and February 2014 under increasing N rates. In the other periods, no effect of N rates on P concentration and content was observed in the diagnostic leaf (Tables 2 and 3).

The effects of N rate on the concentration and content of Ca, Mg and S in coffee trees depended on the evaluated nutrient and period (Tables 2 and 3). Magnesium was constant at all times, with no effect of the increasing N rates on Mg concentration (Table 3). The mean concentrations and contents of Mg indicated a similar response to that found for Ca (Table 4). In February 2013, regardless of the N rate, the mean Mg value was 5.92 g kg^{-1}.

Table 2. Adjusted equations and determination coefficients for the macro- and micronutrient contents of the diagnostic leaf of a conilon coffee tree, according to the applied N rate, in each evaluation period

Nutrient	Period	Fitted equations	R^2/r^2
	E1 (02/2013)	$\hat{Y} = 24{,}0249 + 0{,}0158372*N - 0{,}00000783704*N^2$	0,97
N	E2 (06/2013)	$\hat{Y} = 24.7884 + 0.0241999*N - 0.0000108996*N^2$	0.94
	E3 (10/2013)	$\hat{Y} = 26.9985 + 0.0243265*N - 0.0000133965*N^2$	0.76
	E4 (02/2014)	$\hat{Y} = 24.218 + 0.0177224*N - 0.00000828908*N^2$	0.97
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 1.3429$	-
P	E2 (06/2013)	$\hat{Y} = \bar{Y} = 1.4308$	-
	E3 (10/2013)	$\hat{Y} = 1.53244 - 0.000110994N$	0.91
	E4 (02/2014)	$\hat{Y} = 1.55632 + 0.000286741N - 0.000000307506N^2$	0.71
	E1 (02/2013)	$\hat{Y} = 9.39579 + 0.00212856N - 0.00000129889N^2$	0.84
K	E2 (06/2013)	$\hat{Y} = 8.58356 + 0.00217435N - 0.0000007684N^2$	0.98
	E3 (10/2013)	$\hat{Y} = \bar{Y} = 13.5958$	-
	E4 (02/2014)	$\hat{Y} = 11.9923 - 0.00158737N + 0.000000551408N^2$	0.99
	E1 (02/2013)	$\hat{Y} = 30.3144 - 0.00461332N$	0.78
	E2 (06/2013)	$\hat{Y} = 22.721 + 0.00342559N - 0.0000017233N^2$	0.93
Ca	E3 (10/2013)	$\hat{Y} = 18.1636 + 0.00160476N$	0.94
	E4 (02/2014)	$\hat{Y} = 2.05438 + 0.00350517N - 0.00000158901N^2$	0.96
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 5.9250$	-
	E2 (06/2013)	$\hat{Y} = \bar{Y} = 4.6142$	-
Mg	E3 (10/2013)	$\hat{Y} = \bar{Y} = 3.5917$	-
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 4.4629$	-
	E1 (02/2013)	$\hat{Y} = 2.89078 - 0.000544503N$	0.76
	E2 (06/2013)	$\hat{Y} = \bar{Y} = 1.8596$	-
S	E3 (10/2013)	$\hat{Y} = \bar{Y} = 1.9300$	-
	E4 (02/2014)	$\hat{Y} = 2.51551 - 0.000383616N + 0.000000302709N^2$	0.74
	E1 (02/2013)	$\hat{Y} = 81.5275 + 0.0243552N$	0.83
Fe	E2 (06/2013)	$\hat{Y} = 89.7419 + 0.0399154N$	0.92
	E3 (10/2013)	$\hat{Y} = 95.4434 + 0.0246934N$	0.97
	E4 (02/2014)	$\hat{Y} = 82.8821 - 0.0647851N + 0.0000338768N2$	0.86
	E1 (02/2013)	$\hat{Y} = 10.9887 + 0.00249673N - 0.00000129839N2$	0.98
Zn	E2 (06/2013)	$\hat{Y} = 6.93372 + 0.00164905N$	0.69
	E3 (10/2013)	$\hat{Y} = 6.79272 + 0.00492761N - 0.00000221636N2$	0.94
	E4 (02/2014)	$\hat{Y} = 11.0623 + 0.00111418N - 0.000000607361N2$	0.90
	E1 (02/2013)	$\hat{Y} = 8.70736 + 0.00184989N$	0.72
Cu	E2 (06/2013)	$\hat{Y} = 11.7812 - 0.00942239N + 0.00000461842N2$	0.96
	E3 (10/2013)	$\hat{Y} = 10.4291 - 0.000866808N$	0.97
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 8.2083$	-
	E1 (02/2013)	$\hat{Y} = 59.0562 + 0.0277484N$	0.81
	E2 (06/2013)	$\hat{Y} = 85.1703 + 0.0730749N - 0.0000262746N2$	0.96
Mn	E3 (10/2013)	$\hat{Y} = 52.8204 + 0.0797114N - 0.0000311867N2$	0.99
	E4 (02/2014)	$\hat{Y} = 52.5986 + 0.0790351N - 0.00003341N2$	0.97
	E1 (02/2013)	$\hat{Y} = 91.5678 - 0.00914376N$	0.86
B	E2 (06/2013)	$\hat{Y} = 91.0826 - 0.0269345N$	0.83
	E3 (10/2013)	$\hat{Y} = \bar{Y} = 67.8333$	-
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 101.9167$	-

In general, the increase in N reduced the leaf concentrations and contents of sulfur (Tables 2 and 3). On the other hand, the effects of the N rate on the concentration and content of Fe, Zn, Mn, Cu and B in the coffee trees depended on the micronutrient and the evaluated period (Tables 2 and 3). Iron concentrations were highest in June and October 2013 (109.50 and 107.67 mg kg^{-1}, respectively), with inversely lower concentrations in January 2014 (66.46 mg kg^{-1}). The leaf Mn concentrations were highest in June 2013 (109.21 mg kg^{-1}), differently from Zn, for which leaf concentrations were lower in June of the same year (7.75 mg kg^{-1}). Manganese reached the highest concentrations in June (109.21 mg kg^{-1}) and B in February 2014 (101.92 mg kg^{-1}) (Table 4).

There was a significant effect of nitrogen rates on the concentrations of chlorophyll *a, b* and total chlorophyll in the coffee leaf in all evaluation periods (Figure 1B, C and D; Table 5). The estimated Critical Levels of these variables for

Table 3. Adjusted equations and determination coefficients for the macro- and micronutrient contents of the diagnostic leaf of a conilon coffee tree, according to the applied N rate, in each evaluation period

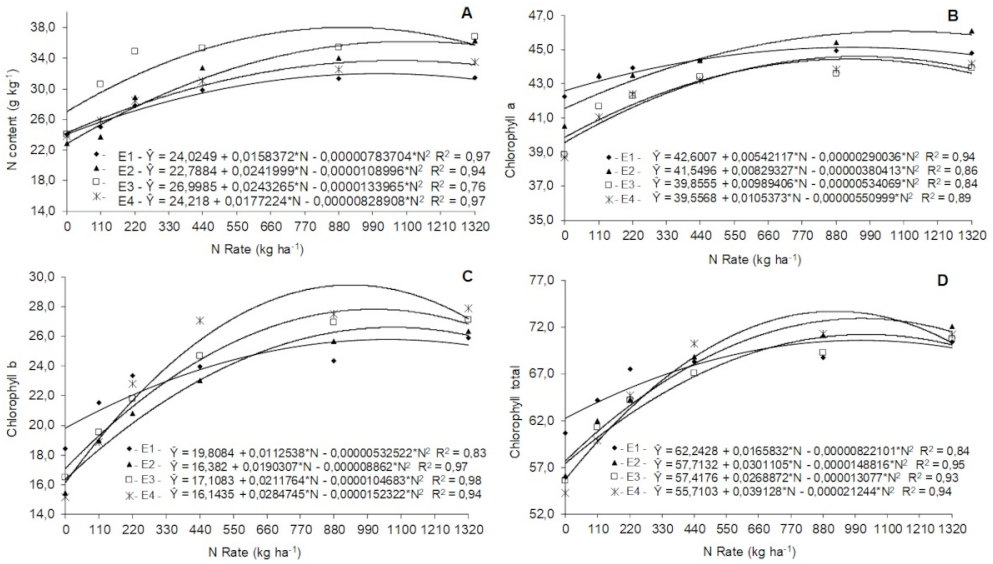
Nutrient	Period	Fitted equations	R^2/r^2
	E1 (02/2013)	$\hat{Y} = 0.0212127 + 0.0000222322N - 0.0000000102547N^2$	0.98
N	E2 (06/2013)	$\hat{Y} = 0.0244509 + 0.0000352892N - 0.000000015838N^2$	0.96
	E3 (10/2013)	$\hat{Y} = 0.0324641 + 0.0000370041N - 0.0000000205528N^2$	0.75
	E4 (02/2014)	$\hat{Y} = 0.02496 + 0.0000391369N - 0.0000000183058N^2$	0.98
	E1 (02/2013)	$\hat{Y} = 0.00122571 + 0.00000014159N$	0.83
P	E2 (06/2013)	$\hat{Y} = \bar{Y} = 0.0017$	-
	E3 (10/2013)	$\hat{Y} = \bar{Y} = 0.0018$	-
	E4 (02/2014)	$\hat{Y} = 0.0016101 + 0.00000143459N - 0.000000000934123N^2$	0.87
	E1 (02/2013)	$\hat{Y} = 0.00832068 + 0.00000463286N - 0.000000000232778N^2$	0.97
K	E2 (06/2013)	$\hat{Y} = 0.0092498 + 0.00000503294N - 0.000000000207551N^2$	0.98
	E3 (10/2013)	$\hat{Y} = \bar{Y} = 0.0170$	-
	E4 (02/2014)	$\hat{Y} = 0.0125048 + 0.00000574836N - 0.000000000316915N^2$	0.92
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 0.0270$	-
	E2 (06/2013)	$\hat{Y} = 0.0244892 + 0.0000105321N - 0.000000000515495N^2$	0.96
Ca	E3 (10/2013)	$\hat{Y} = 0.0215291 + 0.00000796001N - 0.000000000375198N^2$	0.91
	E4 (02/2014)	$\hat{Y} = 0.0213259 + 0.0000181354N - 0.000000000870365N^2$	0.97
	E1 (02/2013)	$\hat{Y} = 0.00496761 + 0.00000237496N - 0.000000000951751N^2$	0.96
	E2 (06/2013)	$\hat{Y} = 0.00494049 + 0.00000230831N - 0.000000000113203N^2$	0.96
Mg	E3 (10/2013)	$\hat{Y} = 0.00424912 + 0.000000915804N$	0.88
	E4 (02/2014)	$\hat{Y} = 0.00456647 + 0.00000318946N - 0.000000000152246N^2$	0.98
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 0.0025$	-
	E2 (06/2013)	$\hat{Y} = 0.00207763 + 0.000000138545N$	0.75
S	E3 (10/2013)	$\hat{Y} = 0.00243616 - 0.00000023792N + 0.000000000020267N^2$	0.81
	E4 (02/2014)	$\hat{Y} = 0.00261839 + 0.00000115689N - 0.000000000424804N^2$	0.98
	E1 (02/2013)	$\hat{Y} = 0.0738356 + 0.0000351365N$	0.88
Fe	E2 (06/2013)	$\hat{Y} = 0.098415 + 0.000058237N$	0.96
	E3 (10/2013)	$\hat{Y} = 0.112097 + 0.0000691429N - 0.0000000245548N2$	0.99
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 0.0794$	-
	E1 (02/2013)	$\hat{Y} = 0.00970812 + 0.00000558445N - 0.000000000259421N2$	0.98
Zn	E2 (06/2013)	$\hat{Y} = 0.00766165 + 0.00000268206N$	0.77
	E3 (10/2013)	$\hat{Y} = 0.00813159 + 0.00000793309N - 0.000000000379969N2$	0.93
	E4 (02/2014)	$\hat{Y} = 0.0115029 + 0.00000868292N - 0.000000000432721N2$	0.97
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 0.0075$	-
Cu	E2 (06/2013)	$\hat{Y} = 0.0127613 - 0.00000792476N + 0.000000000380412N2$	0.95
	E3 (10/2013)	$\hat{Y} = \bar{Y} = 0.0125$	-
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 0.0099$	-
	E1 (02/2013)	$\hat{Y} = 0.0534517 + 0.0000359153N$	0.87
	E2 (06/2013)	$\hat{Y} = 0.0910996 + 0.000113022N - 0.0000000431636N2$	0.97
Mn	E3 (10/2013)	$\hat{Y} = 0.0630539 + 0.000114762N - 0.0000000475014N2$	0.99
	E4 (02/2014)	$\hat{Y} = 0.0532892 + 0.000139376N - 0.0000000597158N2$	0.99
	E1 (02/2013)	$\hat{Y} = \bar{Y} = 0.0839$	-
B	E2 (06/2013)	$\hat{Y} = 0.100618 - 0.0000231475N$	0.84
	E3 (10/2013)	$\hat{Y} = 0.0870766 - 0.0000110444N + 0.000000000655806N2$	
	E4 (02/2014)	$\hat{Y} = \bar{Y} = 0.1239$	-

the periods of December 2012 (E1) and February 2013 (E2) were associated with the N rate that induced a production of 95% of the maximum yield of conilon coffee (420.7 kg N ha^{-1}) in the 1st harvest, while these levels resulted in the same yield percentage when associated with the application of 543.1 kg N ha^{-1} for the 2nd harvest of June 2013 (E3) and February 2014 (E4), (Table 6).

The N rates induced a significant increase in the concentrations of chlorophyll *a, b* and total in the diagnostic as well as the oldest leaves. Regardless of the analyzed leaf, the increase in chlorophyll *b* was higher than that of chlorophyll *a*, especially in the last evaluations (Table 6).

DISCUSSION

The nitrogen concentrations varied between February (summer) and June 2013 (winter), but regardless of the period, they were above the N values found in studies that proposed the establishment of new leaf patterns for conilon coffee in crops with an average yield of more than 100 bags ha^{-1} (11,13,16). This fact was related to the higher N demand with the fruit growth, and the detected differences confirmed that the N concentration of conilon coffee depends on the growth conditions (11), region (12), reproductive cycle (24), genotypes (13 - 15,25), phenological stage (7,9,16) and on seasonality (17).



E1: February 2013; E2: June 2013; E3: October 2013 and E4: February 2014

Figure 1. Estimated contents of nitrogen (A), chlorophyll *a* (B), chlorophyll *b* (C) and total chlorophyll (D) of the diagnostic leaf of a conilon coffee tree, in response to N rates (kg ha^{-1}), in different evaluation periods

Table 4. Means of the macro- and micronutrient contents of the diagnostic leaf of a conilon coffee tree in each evaluation period

Nutrient	Evaluation period			
	E1 (02/2013)	E2 (06/2013)	E3 (10/2013)	E4 (02/2014)
Macronutrient		g kg^{-1}		
Nitrogen	30.69	35.93	40.20	33.84
Phosphorus	1.34	1.43	1.47	1.55
Potassium	9.85	9.30	13.59	11.46
Calcium	28.03	23.62	18.95	21.54
Magnesium	5.92	4.61	3.59	4.46
Sulfur	2.62	1.86	1.93	2.46
Micronutrient		mg kg^{-1}		
Iron	93.58	109.50	107.67	66.46
Zinc	11.62	7.75	8.21	11.33
Copper	7.79	9.25	10.00	8.21
Manganese	72.79	109.21	77.87	76.29
Boron	87.04	77.75	67.83	101.92

Table 5. Adjusted equations and determination coefficients for chlorophyll *a*, *b* or total l of the old leaf of a conilon coffee tree, according to the applied N rate, in each evaluation period

Evaluation period	Fitted equations	R^2
	Chlorophyll *a*	
E1 (December/2012)	$\hat{Y} = 41.8647 + 0.00296553N - 0.00000103622N^2$	0.84
E2 (February/2013)	$\hat{Y} = 40.5182 + 0.0100976N - 0.00000503183N^2$	0.94
E3 (June/2013)	$\hat{Y} = 39.4604 + 0.0101218N - 0.0000054157N^2$	0.87
	chlorophyll *b*	
E1 (December /2012)	$\hat{Y} = 19.0713 + 0.00943109N - 0.00000465222N^2$	0.92
E2 (February /2013)	$\hat{Y} = 16.0898 + 0.0161776N - 0.0000070561N^2$	0.95
E3 (June /2013)	$\hat{Y} = 17.9658 + 0.0179274N - 0.00000894919N^2$	0.92
	Total chlorophyll	
E1 (December /2012)	$\hat{Y} = 59.0668 + 0.0183556N - 0.00000995696N^2$	0.98
E2 (February /2013)	$\hat{Y} = 56.541 + 0.0265501N - 0.0000127586N^2$	0.95
E3 (June /2013)	$\hat{Y} = 57.3799 + 0.0284133N - 0.0000147979N^2$	0.92

Table 6. Estimates of critical concentrations (CLs) associated with the N rate that achieved 95 % of the maximum coffee yield for chlorophyll *a*, *b* or total of the diagnostic leaf and the old leaf, in each evaluation period

Evaluation period	Characteristics					
	Diagnostic leaf			Old leaf		
	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
E1 (December /2012)	44.88	24.54	69.22	43.11	23.04	66.79
E2 (February /2013)	45.04	24.39	70.38	44.77	22.90	67.71
E3 (June/2013)	45.23	28.61	72.02	44.96	27.70	72.81
E4 (February /2014)	45.28	31.61	76.96	-	-	-

The period of highest N demand begins in the flowering phase and is intensified during grain filling, when vegetative growth is also high (7 - 9). The lower N concentrations in leaves collected in February 2014 than October 2013 (Table 4) can be explained by the greater mobilization of N and assimilates to the fruits instead of to the leaves (26). Moreover, heavy rainfall occurred in the region in this period, which also contributed to N leaching into the soil. Nitrogen is an element that is easily lost in the soil-plant system by leaching, volatilization and denitrification, which hampers an adequate management of N fertilization (27).

Regardless of the N rates, the P content was within the sufficiency range determined for the region (11,13), in spite of reductions in the P and K concentrations in October 2013 and February 2014 in response to increasing N rates. However, considering the high mobility of P in the plant, the fruit sink strength is one of the main factors that can influence P concentration in the coffee leaves; in this study, the increase in conilon coffee yield in response to increasing N rates resulted in lower P concentrations and content in the diagnostic leaf. In other words, the greater the fruit load in response to higher N rates, the greater are the fruit sink strength and nutrient partition, regardless of higher soil N concentrations.

On the other hand, the K concentrations (Table 4) were below or at the lower limit of the sufficiency ranges proposed by (13), except in the sampling carried out in October 2013, when the mean value (13.59 g kg^{-1}) was within the range proposed for winter sampling and reached 11.46 g kg^{-1} in February 2014. As mentioned for nitrogen, potassium can be lost by leaching, due to high rainfall, and reduced adsorption to soil particles. Potassium availability is determined by soil moisture conditions, where leaching occurs in response to the K content in the soil solution and the amount of water percolating through the profile, which may have contributed to the reduced K availability near the crop uptake zone and consequently in the leaves.

The lower K uptake in this study may be explained by the occurrence of competition with other macronutrients (Ca and Mg), by competitive inhibition (10). This increase in Ca and Mg uptake could explain the lower K uptake by coffee trees, as observed in this study, due to the antagonism between these elements.

The effects of the N rate on the concentration and content of Ca, Mg and S in the coffee trees depended on the nutrient and the evaluated period (Tables 2 and 3) (28), suggesting a synergistic effect of higher N rates on nutrient uptake, in particular of Ca (6) . Higher Ca concentrations in different periods were also observed by (13,16) and as in this study, indicated that a higher Ca accumulation in the leaves of coffee plants confirms the characteristically low mobility of this nutrient in the plant, which is not efficiently relocated from the leaves to the fruits (26).

The N rates did not change the Mg macronutrient content but were constant at all times (Table 3). High Ca concentrations can inhibit Mg uptake, decrease its root-to-shoot translocation and thus, cause Ca deficiency. The reason is that Ca and Mg compete for the same uptake sites in the root, causing a preferential uptake of the cation with highest concentration in the soil solution at the expense of the others.

When calcium and potassium are applied to the soil, they compete effectively with Mg, resulting in induced deficiency. Magnesium deficiency induced by competitive cations is a relatively frequent phenomenon. The higher amount of P in the tissue is also an indicator that Mg acts as a phosphorus carrier. The increasing N rates in this study were favorable for nutrient accumulation for increasing the biomass production and stimulating vegetative and root growth, which resulted in greater Mg uptake. In addition, the literature suggested a synergistic effect of higher N rates on Mg uptake (6) .

Despite the reductions in leaf S concentration and content in response to increasing N rates, the S concentrations were within or above the ranges considered appropriate for the region (13) (Tables 6). In other crops, the reduction in tissue concentrations of this macronutrient is common, due to reduced uptake of sulfate under higher nitrate and phosphate concentrations, due to the competition by the same uptake mechanisms in the plasma membrane. The combined result of these factors is a decrease in plant S concentrations as the N rates and P in the soil and their concentrations in the leaf tissues rise, mainly under the influence of the fruit sink strength and greater nutrient distribution. In addition, the reduced leaf S content may be due to leaching beyond the root tips.

There was an increase in the concentration and content of Fe, Zn, Mn and a reduction in Cu and B under increasing N rates, with fluctuations in relation to the evaluation periods. Nevertheless, despite these responses, all micronutrients were within the appropriate range proposed for the region (13).

The changes recorded in this study result from a peculiar physiological period of coffee, characterized by intense cell

division and high respiratory rates, when micronutrients become more concentrated due to the lower fruit biomass high (7,19,29). Leaf concentrations and contents also varied during the evaluation period (Table 6). The Fe, Cu and Mn concentrations reached minimum levels in the physiological period preceding the fruit ripening phase (19).

The increase in Fe concentration and content under increasing N rates in this study may be attributed to the higher Ca concentrations that favor micronutrient uptake, among them Fe, and to the greater plant growth induced by N, also intensifying an increased Fe uptake. In addition, Fe and Mn were positively correlated, indicating that the accumulation of one favors the accumulation of the other by the plant, i.e., they have a synergistic interaction.

The directly proportional relationship between Zn and increased N rates may also be related to the increased Ca content, since the relevance for the functional membrane stability of Ca leads to an increase in micronutrient contents, among them Zn. In addition, the period of greatest Zn demand for conilon coffee coincides with the period of greatest vegetative growth (7,30). This overlapping may lead to a competition for nutrients between reproduction and vegetative growth in other plant parts (24).

The increase in Mn concentrations in response to increasing N rates showed a positive correlation between Fe and Mn, indicating that the accumulation of one favors the accumulation of the other by the plant, i.e., they reflect a synergistic interaction. Manganese ions together with Fe are responsible for the activation of a number of enzymes in plant cells, as well as photosynthetic processes and hydrolysis (31).

The reduction in B concentrations in the diagnostic leaf under increasing N rates in this study may be related to the yield increase, with an increased flower and fruit production, allocating a greater amount of B to these organs. Nevertheless, and regardless of the N rate, the B concentrations in the sampling of February 2014 were higher than in October 2013 (Table 4), probably due to the low B mobility. The demand for this nutrient is particularly high in the pre-flowering phase, which explains the responses to B application in this phase. According to (7,32), in the initial fruit growth ("chumbinho") stage, the proportion of B accumulation in the fruits was higher than that of other micronutrients. This is related to the high relevance of this nutrient in processes of cell division and membrane stabilization of the newly formed cells.

The N rates influenced the concentrations of chlorophyll *a, b* and total in the diagnostic as well as the oldest leaves. These data are relevant for showing a direct relationship with N fertilization and confirming the use of a chlorophyll meter as an important auxiliary tool to detect possible changes in N levels. In addition, in this way, data of the nutritional diagnosis of nitrogen in *C. canephora* can be recorded quickly and effectively (18,20).

The use of the diagnostic leaf for indirect measures of the chlorophyll content is more appropriate than of the oldest leaf, since the former is considered physiologically more active. In addition, the physical access to the diagnostic leaf on the plant is easier, making field measurements faster, while older leaves are more likely to have been affected by biotic or abiotic stress.

In this study, the evaluation sites on the plant influenced the chlorophyll readings in response to the different N rates. Younger leaves (diagnostic leaf) have a greater chlorophyll synthesis capacity (31) and therefore a higher "green" intensity, with consequently higher values of indirect readings (Table 6).

The nitrogen concentration increased gradually after June, reaching maximum values in the October period, followed by a decline in February (Figure 1A, Table 2). Similar results were found by (17) for conilon and by (33) for Arabica coffee. In both studies, the leaf N concentration declined with the age of the sampled plant. This variation in N concentration can be explained by mechanisms related with N uptake, accumulation and distribution in the plant, as well as the plant development. In this context, a standardization of the leaf to be used in the diagnostic analysis is required, since the chlorophyll index measures the chlorophyll content in the plant indirectly, determining the nitrogen nutritional status at a specific stage of the crop cycle.

Thus, the use of N indices as an indirect measure of the chlorophyll content, which is sensitive to N applications, easily determinable and non-destructive, can also be used as an indirect index for the diagnosis of the nitrogen nutritional status in conilon coffee.

CONCLUSIONS

- The N rates influenced the cumulative concentrations of N, P, K, Ca and S as well as Fe, Zn, Mn, Cu and B in the coffee leaf and depended on the nutrient and the evaluated season. The macronutrient concentrations in the leaves of the coffee plant were highest in the evaluation period of June.
- The use of indirect measurements of chlorophyll content can be an important tool to diagnose the nitrogen status in conilon coffee, and the measurement of the diagnostic leaf is recommended, due to the easy physical access to it on the plant, ensuring a faster field measurement.

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