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Concentration and accumulation of macronutrients in leaf of coffee berries in the Amazon, Brazil

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Abstract

Knowledge regarding the behaviour and accumulation of nutrients in coffee berries and leaves is important information for the management of fertilisation. Therefore, this study aimed to evaluate the concentration and accumulation of macronutrients in coffee fruits and leaves under different fertilisation regimes in the south western Amazon, Brazil. The experiment was conducted in of plots subdivided by time: the main plots consisted of two fertilisation regimes (fertilised and unfertilised plants), and evaluation periods were in subplots (leaves: May 2013 to June 2014, fruits: July 2013 to April 2014). The experimental design was of randomised blocks with three replicates. Each experimental plot consisted of 11 plants; two productive plagiotropic branches were marked on each plant. Fruits were sampled every 28 days from the "pinhead" stage (July 2013) to maturation (April 2014). Twenty leaves were concomitantly sampled from each block until June 2014. The berries and leaves were dried in a convection oven and sent for laboratory chemical analysis. It was found that mineral fertilisation affects the concentration and accumulation of nitrogen, phosphorus, potassium, calcium and magnesium in berries and leaves. The concentration of macronutrients at the beginning of fruit formation is high and tends to decrease at later stages. In leaves, lower concentrations occur at the stage when the fruit is small and increase with growth. Most of the accumulation occurs in the expansion, bean formation and maturation stages the fruits.

Keywords: *Coffea canephora*, macronutrients, accumulation curve, nutritional management, reproductive stage, south western Amazon.

Introduction

Coffee is an important commercial crop cultivated in approximately 50 developing countries around the world. Millions of people depend directly or indirectly on the production and sale of coffee for their livelihood and income (Bagyaraj et al., 2015). *Coffea arabica* and *Coffea canephora* are the species of greatest economic importance within the *Coffea* genus, which includes more than 120 species (Davis et al., 2011). In the Brazilian Amazon, coffee crops are mainly composed of *C. canephora* because the conditions for its cultivation are appropriate. It is known, there is great potential for increasing production, but productivity levels are low and have not significantly increased in the recent years due to several factors, among which suboptimal crop management stands out. Among the main obstacles related to management that hinder high productivity, is the mineral nutrition because coffee plant requires abundant nutrients for fruit formation and vegetative growth (Laviola et al., 2007c; Partelli et al., 2014). The macronutrients most required by the *C. canephora* and that are mostly accumulate most are nitrogen > calcium > potassium > magnesium > sulphur > phosphorus, in that respective order (Bragança et al., 2008).

During the reproductive stage of coffee plants, the fruits go through different phenological stages. They start with the flowering and then proceed to fruit development, which is composed of the pinhead, rapid expansion, bean formation and maturation stages (Cunha et al., 2011; Partelli et al., 2014). Each formation stage has particular physiological and metabolic functions essential to the final formation of this organ, and consequently, there are variations in the concentration and type of elements accumulated at each stage; that is, there are periods that are less demanding of nutrients, and others are more demanding (Laviola et al., 2007b, c; Partelli et al., 2014). In the pre- and post-flowering stages, nutrient absorption is efficient and high levels are present in inflorescences, which therefore act as a strong temporary drain on the plant. Thus, before the flowering stage, the plant must be well nourished to ensure successful flowering and consequently successful fruit production (Malavolta et al., 2002; Valarini et al., 2005). In the fruit formation period, higher accumulation rates of major macronutrients (nitrogen, phosphorus and potassium) are found in the rapid expansion stage, bean formation and maturation stages of Arabica coffee berries, being highest in the maturation stage. As for secondary macronutrients (calcium, magnesium and sulphur), the highest relative accumulation rates occur in the stages of bean formation and maturation (Laviola et al., 2007a, 2008). Thus, berries are the preferred drain in the partition of nutrients during the fruiting stages and the higher the berry production, the higher the demand for nutrients; therefore, attention to nutrition is required at this important stage of the coffee plant. Thus, nutrient delivery must be performed before the peak of element accumulation in the fruit; it must also meet the nutrient demands for vegetative growth (Partelli et al., 2013), which mostly occurs concomitantly with the coffee reproductive stage (Laviola et al., 2007b; Partelli et al., 2014).

Knowledge regarding nutrient accumulation curves in coffee fruits is an important tool for estimating the nutritional needs of the crop; allowing the periods when the plant needs nutrients the most and the proper time for application of fertiliser to be identified, thus providing better utilisation, avoiding losses and increasing the efficiency of the applied product (Ramirez et al., 2002). In addition, it is very valuable to know the variations in nutrient content in leaves and nutrient mobilisation to fruits during the reproductive stage of the coffee plant due to the source-sink effect that occurs due to the mobility of nutrients and their requirement for the formation of the fruit.

Studies have been conducted in the main coffee regions (Espírito Santo, Minas Gerais, São Paulo and Bahia); however, in the Amazon region, with its distinct edaphoclimatic characteristics, knowledge is still lacking regarding the dynamics of nutrients in the fruits and leaves of this crop. Thus, this study aimed to evaluate the concentration of macronutrients in fruits and leaves and their accumulation in coffee berries under different fertilisation regimes in the south western Amazon.

Results and Discussion

Fertilisation regimes

It was found that the fertilisation promoted significant effects on the concentration of nutrients in coffee berries for nitrogen and calcium only. Different behaviour was seen in leaves, where fertilisation influenced the concentrations of nitrogen, phosphorus, potassium and magnesium only in certain periods. The concentration in fruits and leaves of all the macronutrients was significantly influenced by the sampling time of the plant tissue (Table 2). The average nitrogen concentration was higher in berries of plants that received fertilisation than in unfertilised berries (Table 2). Thus, it was verified that nitrogen fertilisation is efficient and the coffee plant is responsive to it, as this nutrient accumulates at a greater proportion in the whole plant compared to the others (macro and micronutrients) (Bragança et al., 2008). In addition, nitrogen assimilation is an essential process that controls the growth and development of plants, and it has remarkable effects on biomass and crop yield (Oaks, 1994). Moreover, nitrate acts in signalling for the local control of root development, providing increased emission of lateral roots (Lima et al., 2002).

Behaviour inverse to that of nitrogen occurred for calcium, where fruits with no fertilisation accumulated a higher content than fertilised fruits. These results can be explained because high concentrations of K^+, Mg^{2+} and NH_4^+ in the soil can decrease the absorption of Ca^{2+}, which is the form of this nutrient that is absorbed by the plant roots (Vitti et al., 2006). A reverse effect may also occur when there are high concentrations of exchangeable Ca in the soil, inhibiting the absorption of Mg^{2+} and K^+ (Medeiros et al., 2008). Another plausible explanation for this calcium behaviour may be a dilution effect of the nutrient's concentration caused by the higher growth of berries in fertilised plants, mainly due to the addition of nitrogen (Prezotti et al., 2013).

Ionic calcium is used in the synthesis of new cell walls, especially the middle lamella separating newly divided cells. It is necessary for the good normal operation of membranes, and it also has a secondary messenger role in various plant responses such as environmental and hormonal signals (Taiz & Zeiger 2010). In addition, it operates in plant defence responses to pathogens, as higher calcium concentrations are observed in symptomatic areas of coffee plant leaves with bacterial blight, Cercospora and Phoma leaf spot. The displacement of calcium to necrotic areas occurs as a defence reaction, especially when there is rupture of membranes, because it is essential in the formation of structural and chemical barriers (Belan et al., 2015).

Concentrations of macronutrients in leaves and fruits

The concentrations of macronutrients in leaves differed between treatments from the post-flowering period until post-harvesting, and there was a significant interaction between the nutritional regime and the period evaluated for the nutrients nitrogen, phosphorus, potassium and magnesium (Tables 2 and 3). For nitrogen, the fertilised plants attained higher mean leaf concentrations in early December, late March and May, but in June, this situation was reversed, with higher concentrations in leaves of plants that were not fertilised. Phosphorus showed a higher mean concentration in late January and inverse behaviour in late April. For potassium, a higher concentration occurred in late February and a lower concentration in late June. Higher concentrations of magnesium were observed in late January and lower concentrations occurred in early December, late March and April.

Fluctuations in leaf concentrations can occur due to the different nutritional requirements of the fruits during their cycle because each stage has its own requirements and the nutrient demand tends to be higher in the expansion, bean formation and maturation stages (Prezotti et al., 2013; Partelli et al., 2014). For particular cases such as magnesium, which showed higher concentrations in most periods in the plant leaves without fertilisation, the cause can be attributed to other cations such as K^+, NH_4^+, Ca^{2+} and Mn^{2+} present in the soil affecting the absorption of this nutrient (Vitti et al., 2006), thus causing low concentrations in the leaves of plants that were fertilised.

Macronutrient concentrations in both the fruits and leaves showed variation throughout the evaluation period; therefore, it was not possible to establish trend lines (Figure 2). Higher concentrations of nitrogen, phosphorus, calcium, magnesium and sulphur were found in the initial stage of fruit formation. Specifically, nitrogen (July) showed higher concentrations in the first sampling compared to the rest of the evaluation period. As for phosphorus, calcium, magnesium and sulphur, they showed less significant concentrations in July and increased in the following months, with P reaching its highest proportion of the entire evaluation period in October, calcium and magnesium in September and sulphur in August (Figure 2). This period is known as the pinhead stage, where the berry is still small in size and has a low accumulation of dry matter, contributing to the concentration of elements in the fruit (Partelli et al., 2014).

Table 1. Chemical analysis results of the soil in the experimental area at different depths.

Sample	pH in water	P mg dm^{-3}	K	Ca	Mg mmolc dm^{-3}	Al+H	Al	MO g kg	V %
00-10 cm	7.2	86	19.23	66.1	17.2	18.2	0.0	34.5	85
10-20 cm	7.3	45	8.21	69.7	8.4	11.6	0.0	17.8	87
20-30 cm	6.9	13	5.03	41.8	7.6	24.8	0.0	17.8	69
30-40 cm	6.7	3	6.41	26.2	6.6	16.5	0.0	16.1	70

pH (hydrogenionical potential): in H2O 1:2.5; P and K (phosphorus; potassium): Extraction: Mehlich 1; Ca and Mg (calcium; magnesium): Extraction: KCI 1 mol/L; Al+H (aluminium, hydrogen): Titration; MO (organic matter): Embrapa Method; V% (base saturation).

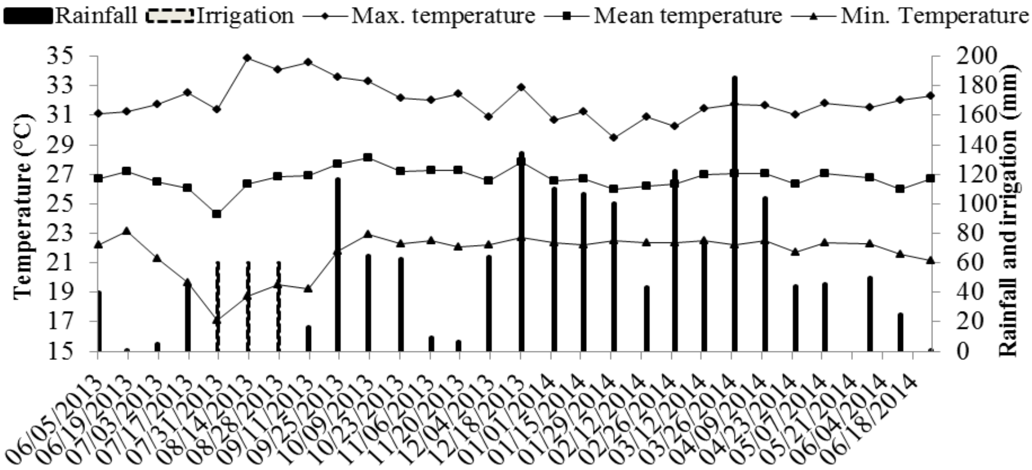


Fig 1. Rainfall; irrigation; and minimum, mean and maximum temperature during the experimental period. South Western Amazon 2013/2014.

These results are in agreement with those found by Laviola et al. (2006, 2007 a, c, d) who, assessing the dynamics of macronutrients in Arabica coffee cultivars at three levels of fertilisation, found that the maximum concentrations of these nutrients occurred in the pinhead stage. After this peak accumulation, there was a drop in the nutrient concentrations in question, with several fluctuations, reaching the lowest values at the expansion, bean formation and maturation stages. This behaviour is due to the increased dry matter content in the fruit; that is, the amount of nutrients present in the berry is diluted. The expansion, bean formation and maturation stages are characterised by cell expansion, an increase in size and the accumulation of reserve substances in the fruit (Laviola et al., 2006). Obtained similar results Laviola et al. (2006; 2007 a, c, d), for concentration nitrogen, phosphorus, calcium, magnesium and sulphur in Arabica coffee cultivars. Potassium behaved differently from the other nutrients, and it was noticeable that the concentration of this element was higher in the fruit than in leaves, confirming the importance of this nutrient in the formation of fruit. Potassium is active in many physiological processes, promoting the activation of more than 60 enzymatic systems, participating in photosynthesis, favouring a high energy state, maintaining cell turgor, regulating the opening and closing of stomata, promoting water absorption, regulating the translocation of nutrients, favouring the transportation and storage of carbohydrates, increasing the absorption of nitrogen and proteins and participating in starch synthesis in leaves (Taiz & Zeiger 2010). The potassium concentration showed no significant differences with respect to the time of fruit sampling; there were small fluctuations throughout the period and a slight trend of higher content in the pinhead stage. Similarly, Laviola et al. (2006) found changes in potassium concentration in fruit, and the highest concentration was observed up to 42 days after floral anthesis for the evaluated varieties. The leaf concentrations behaved very differently from those of the berries, and the different nutrients behaved differently. Nitrogen, phosphorus and sulphur concentrations were at their lowest at the beginning of the sampling, increasing in the following months, but with fluctuations. As for nitrogen, similar results have been found at this stage in leaves of three Arabica coffee cultivars with low concentrations up to 42 days after floral anthesis (Laviola et al. 2006). In a different situation, lower nitrogen content was reported in cv. Catuaí leaves sampled in January 2013 grown under high CO_2 concentration, and this behaviour may be attributed to the greater growth of the coffee plant in this condition causing dilution of the nutrient (Ghini et al., 2015). As for phosphorus, different data were found, where the content increased until a maximum concentration was achieved in the middle of the fruit development stages, with a subsequent decrease up to 224 days after flowering. A lower concentration of sulphur was also found at the pinhead stage under high fertilisation, and higher contents were found in the bean formation/maturation stage (Laviola et al., 2007d). This inverse behaviour observed for nitrogen and phosphorus between leaves and fruit, mainly at the initial period of evaluation, may be because of the source-sink effect occurring from leaves to fruit due to the nutrients required for fruit formation at this stage, as nitrogen and phosphorus are high-mobility nutrients that promote this phenomenon. Lima Filho & Malavolta (2003), Laviola et al. (2006, 2008) report that when the absorption of nitrogen through soil is insufficient for bean formation, the remobilisation of nitrogen from leaves then occurs to meet the required demand. The mobilisation of phosphorus from leaves to fruits also tends to increase as a function of the amount of fruit produced; that is, greater production results in lower leaf concentrations of this element (Laviola et al., 2007d). The nutrients calcium and magnesium behaved in the opposite way, with higher concentrations in the first assessments and lower concentrations during the expansion and bean formation stages. Fluctuations in calcium concentration in leaves of three Arabica coffee cultivars were found by Laviola et al. (2007c), and the initial stage showed higher concentrations. Different results were observed by Valarini et al. (2005), with

Table 2. Summary of the analysis of variance (ANOVA) for the concentration of macronutrients in post-blooming fruits and leaves until maturation and average concentration in fruits.

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur
Source of variation	F value					
	Fruit Concentration					
Fertilisation man. (a)	41.76**	1.53^{ns}	0.01^{ns}	10.71*	1.87^{ns}	1.14^{ns}
Evaluated time (b)	18.79**	14.50**	1.51^{ns}	33.16**	25.74**	28.35**
axb interaction	0.89^{ns}	0.73^{ns}	0.88^{ns}	1.06^{ns}	0.36^{ns}	1.24^{ns}
	Leaf Concentration					
Fertilisation man. (a)	7.18^{ns}	7.01^{ns}	0.20^{ns}	0.12^{ns}	1.67^{ns}	0.03^{ns}
Evaluated time (b)	6.53**	14.81**	9.78**	15.25**	16.76**	2.70**
axb interaction	2.42*	2.49*	6.58**	1.17^{ns}	4.06**	1.20^{ns}
TREATMENT	Average concentration in fruits (g kg-¹)					
Fertilised plants	32.97a	1.86a	26.30a	7.09b	2.16a	2.51a
Unfertilised plants	28.70b	1.93a	26.18a	8.26a	2.36a	2.69a
CV%	8.71	13.42	16.34	18.93	26.89	26.82

*, **, ns, significant at the 1% level, significant at the 5% level, and not significant, respectively. Means followed by the same letter do not differ statistically by Tukey's test at 5% probability.

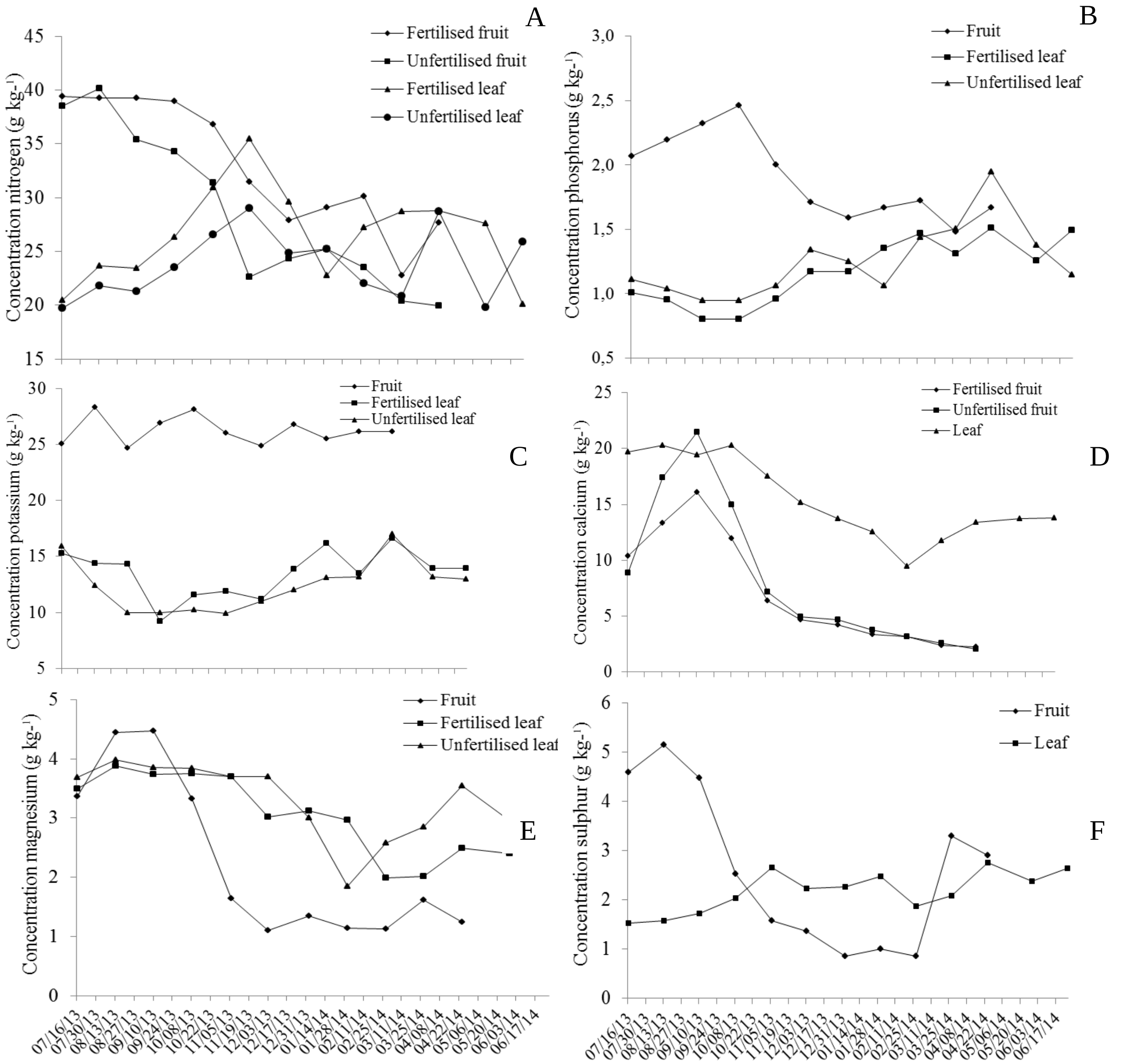


Fig 2. Concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur in the fruit and leaves of *C. canephora* subjected to two fertilisation regimes (fertilised and unfertilised) sampled from the pinhead to maturation stages and at post-harvesting. South Western Amazon 2013/2014.

Table 3. Concentration of nutrients (g kg-¹) in the leaves of the coffee plant *C. canephora,* fertilised and unfertilised, during the post-flowering period (July 2013) until post-harvesting (June 2014).

Collec-tion dates	Nitrogen (g kg-¹)		Phosphorus		Potassium		Calcium		Magnesium		Sulphur	
	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
07/16/13	20.4a	19.7a	1.0a	1.1a	15.3a	15.9a	19.0	20.2	3.4a	3.6a	1.6	1.4
08/13/13	23.6a	21.7a	0.9a	1.0a	14.4a	14.1a	21.1	19.3	3.8a	3.9a	1.5	1.5
09/10/13	23.4a	21.2a	0.8a	0.9a	14.3a	12.4a	19.2	19.6	3.7a	3.8a	1.6	1.8
10/08/13	26.3a	23.4a	0.8a	0.9a	9.2a	10.0a	20.4	20.1	3.7a	3.8a	1.9	2.1
11/06/13	30.9a	26.5a	0.9a	1.0a	11.6a	10.2a	17.7	17.2	3.7a	3.7a	3.1	2.2
12/03/13	35.4a	29.0b	1.1a	1.3a	11.9a	9.9a	14.8	15.5	3.0b	3.7a	2.6	1.8
01/02/14	29.6a	24.8a	1.1a	1.2a	8.7b	18.8a	12.2	15.2	3.1a	3.0a	2.1	2.3
01/30/14	22.7a	25.1a	1.3a	1.0b	13.9a	12.0a	14.8	10.2	2.9a	1.8b	2.2	2.6
02/27/14	27.1a	22.0a	1.4a	1.4a	16.2a	13.1b	8.5	10.3	1.9a	2.5a	2.1	1.6
03/27/14	28.7a	20.8b	1.3a	1.5a	13.5a	13.2a	11.8	11.7	2.0b	2.8a	2.1	2.1
04/24/14	28.8a	28.6a	1.5b	1.9a	16.6a	17.0a	12.1	14.7	2.4b	3.5a	2.1	3.2
05/29/14	27.6a	19.7b	1.2a	1.3a	13.9a	13.1a	12.8	14.5	2.3a	2.9a	2.5	2.1
06/26/14	20.1b	25.9a	1.4a	1.1a	13.9b	17.5a	13.9	13.6	3.0a	2.4a	2.5	2.7
Mean	26.5a	23.7a	1.1a	1.2a	13.3a	13.6a	15.2a	15.5a	3.0a	3.2a	2.1a	2.1a
CV%	18.04		9.89		21.46		23.23		20.36		27.80	

F and NF, fertilised and unfertilised. Means followed by the same letter in the same column do not differ statistically by Tukey's test at 5% significance.

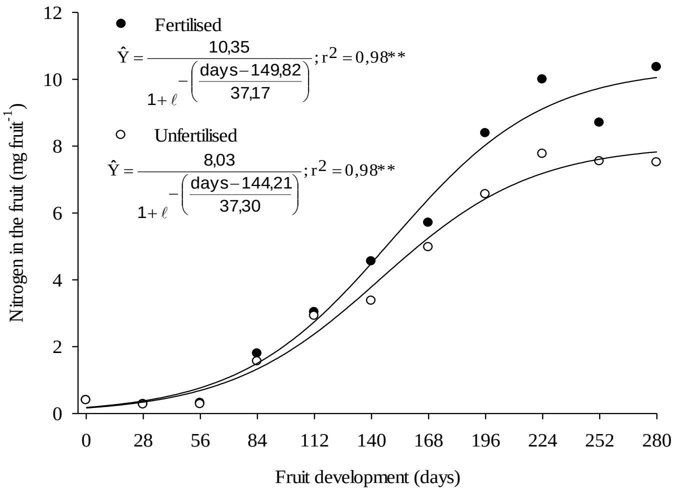


Fig 3. Accumulation of nitrogen in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.

considerable increases of calcium content in leaves of Arabica coffee cultivars in December, February, and May of 12.6, 16.3 and 19.9 g kg^{-1}, respectively. As for magnesium, completely opposite results were found by Laviola et al. (2007c); at the initial stage (pinhead) the authors reported low concentrations of this nutrient, which increased in the following stages (expansion, bean formation and maturation). Remobilisation of calcium from leaves to fruit tends to be unusual, as seen in these results, because the calcium contained in leaves has low mobility due to low concentrations in the symplasm and phloem, and it can be redistributed only under special conditions, such as injection of other cations into the ribs or treatment with triiodothyroacetic, triiodobenzoic, malic or citric acid (Vitti et al., 2006).

In contrast, potassium occurred at higher concentrations in the initial and final stages of the evaluation, and lower concentrations occurred in the expansion and grain formation stages. Similar to the results of Valarini et al. (2005) in Arabica coffee plants grown in the state of São Paulo, there was a decrease in potassium content in leaves from December to May.

Accumulation of macronutrients in the fruit

When the accumulation of macronutrients was assessed in the fruit, a significant effect was found only for nitrogen due to the fertilisation regime applied. For the other nutrients, the treatments did not increase their accumulation in the fruit (Table 4). There was no interaction between fertilisation management and time of evaluation for any of the nutrients.

When the treatment averages are compared, it can be seen that fruits from fertilised plants accumulated more nitrogen than fruits from unfertilised plants (Table 4), and the same behaviour was observed for concentration in the fruit (Table 2). Nitrogen stood out as the most accumulated nutrient in the coffee plant berry, with a mean of 4.76 mg berry^{-1}, behind potassium only (Table 5). This shows the importance of this nutrient and its function in the formation of the fruit, showing the benefits of nitrogen fertilisation for the coffee crop during the reproductive period. According to Clemente et al. (2011), nitrogen is more important for the production of branches and the vegetative growth of the coffee plant, but it also has essential functions in fruit formation. The nitrogen accumulation in the fruit at the initial stage (approximately 60 days after flowering) was low (Figure 3). In this period, this

Table 4. Summary of analysis of variance (ANOVA) for the accumulation of nitrogen, phosphorus, potassium, calcium, magnesium and sulphur in coffee plant berries from the pinhead stage until maturation and average accumulation.

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur
	F value					
Fertilisation man. (a)	94.28**	0.14^{ns}	0.36^{ns}	0.97^{ns}	0.31^{ns}	0.19^{ns}
Evaluated period (b)	71.59**	94.52**	201.19**	46.76**	19.26**	41.68**
Interaction (axb)	1.43^{ns}	0.13^{ns}	0.79^{ns}	1.21^{ns}	0.33^{ns}	1.14^{ns}
Average accumulation macronutrient						
Fertilised plants	4.85 a	0.28 a	4.49 a	0.62 a	0.23 a	0.34 a
Unfertilised plants	3.91 b	0.28 a	4.37 a	0.64 a	0.21 a	0.33 a
CV%	8.99	27.01	17.76	13.59	46.67	21.68

** significant at 1%, ^{ns} not significant. Means followed by the same letter do not differ from each other.

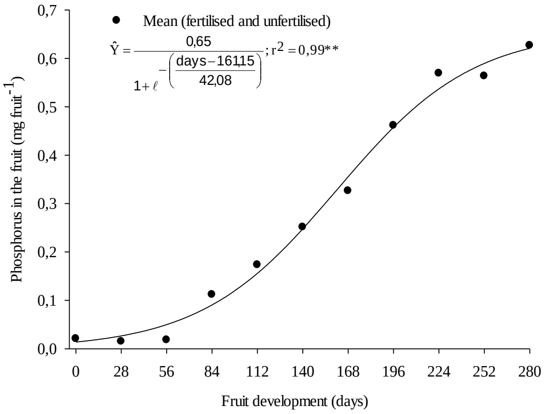


Fig 4. Accumulation of phosphorus in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.

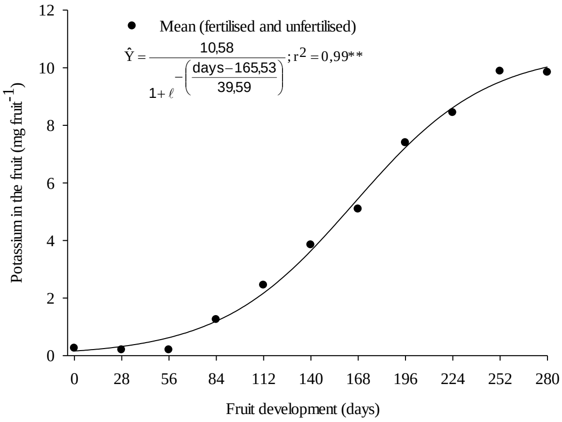


Fig 5. Accumulation of potassium in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.

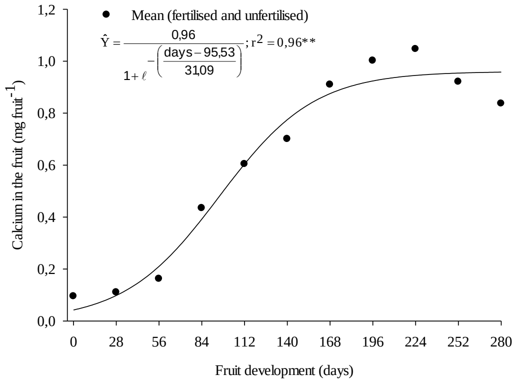
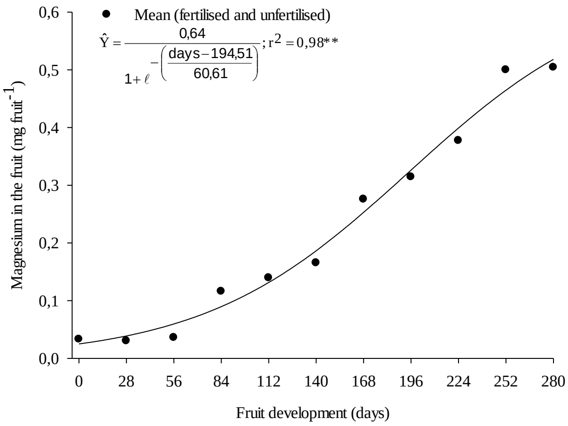
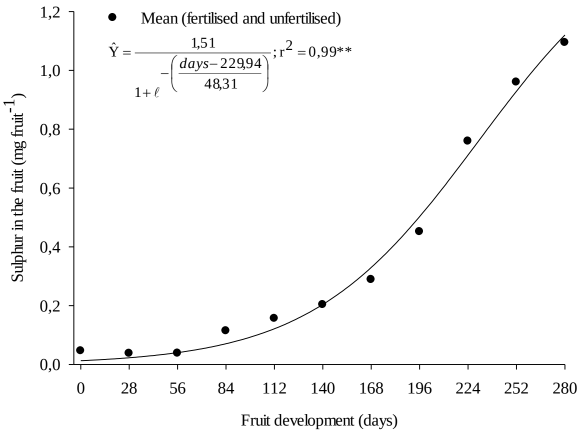


Fig 6. Accumulation of calcium in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.



$$\hat{Y} = \frac{0,64}{1+\ell^{-\left(\frac{days-194,51}{60,61}\right)}};\ r^2 = 0,98^{**}$$

Fig 7. Accumulation of magnesium in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.



$$\hat{Y} = \frac{1,51}{1+\ell^{-\left(\frac{days-229,94}{48,31}\right)}};\ r^2 = 0,99^{**}$$

Fig 8. Accumulation of sulphur in coffee plant berries sampled from the pinhead stage until maturation. South Western Amazon 2013/2014.

behaviour can be attributed to the berries being in the pinhead stage, characterised by low growth and accumulation of dry matter, therefore reducing the accumulation of nutrients in tissue (Laviola et al., 2006, 2007a, 2008). However, Partelli et al. (2014), in early and intermediate maturity genotypes of the conillon species, verified an increase in nitrogen accumulation after the second evaluation month (September).
In the period from October to February, the nitrogen accumulation presented sigmoidal behaviour, peaking in December and remaining stable in the following two months. At those times, the coffee fruit is in the rapid expansion, bean formation and maturation stages. These stages are characterised by rapid cell elongation, endosperm filling and increasing sugar content (Laviola et al., 2007b); that is, in this period, there is high nutritional demand for full fruit formation. Similar results were found by Laviola et al. (2008) with the highest nitrogen accumulation rates in the rapid expansion, bean formation and maturation stages. The same was found by Partelli et al. (2014) in late and super late cycle genotypes of conillon coffee clones in Espírito Santo, which were different from early and intermediate clones. The accumulation of phosphorus also presented a sigmoidal shape, and in the pinhead stage, the period between July and early September, there was no significant accumulation of this nutrient in the fruit (Figure 4). In the following month (October), increments of 0.11 mg berry^{-1} were observed, gradually increasing until the last evaluation month (April), peaking at 0.62 mg berry^{-1}. Note that the highest accumulations are concentrated in the expansion, bean formation and maturation stages of the fruit, confirming the nutritional need for fruit formation in this stage. This same behaviour was found by Laviola et al. (2008) in Arabica coffee plant berries and Partelli et al. (2014) in conillon coffee plant berries of super late cycle, with maximum accumulation rates at those three stages, and because the early, intermediate and late clones displayed significant accumulation rates from the initial stage, this behaviour occurs as a function of the reduced time for fruit formation.
As for potassium, there was no significant accumulation initially in the period between July and September. Similar results were found by Laviola et al. (2008) in Arabica coffee and Partelli et al. (2014) in a super-late clone of conillon coffee in Espírito Santo, with zero accumulation of potassium in the pinhead stage. In the pinhead stage, the low growth rates and dry matter accumulation may be due to the high respiratory rate and intense cell multiplication (Laviola et al., 2007b). In the first sampled pinhead berries (July), a small increase of potassium was more significant (0.25 mg berry^{-1}) compared to the two subsequent evaluations (0.19 mg). Such behaviour can be explained because, in this period, the flowers on the branches were placed with the berries for the analysis. In research conducted by Malavolta et al. (2002), it was found that among the nutrients, potassium had the highest content in this organ, with approximately 80 kg ha^{-1}

of this nutrient extracted, consisting of a strong temporary nutrient sink with higher contents than the leaves and branches. After October, the rates showed significant increases, extending until March. In this period the fruits go through the rapid expansion, bean formation and maturation stages; that is, they grow in size and weight, and thus, they need greater amounts of potassium due to its role in the formation of starch (starch synthesis activation), which is essential for coffee production (Bragança et al., 2008). Similar behaviour was observed by Partelli et al. (2014) in conillon coffee plants, with maximum accumulation rates of potassium in these three stages of the coffee plant reproductive cycle. In Costa Rica, Ramirez et al. (2002) observed a reproductive cycle of 240 days for coffee plants, where 93% of the total potassium was accumulated in the last 180 days, that is, from the third month after flowering until fruit maturation, showing very similar behaviour with the study in question.

Constant potassium accumulation until maturation was observed by Laviola et al. (2008) in Arabica coffee berries, who attributed the cause to the nutrient being required for the activation of several key enzymes in the synthesis of organic compounds that are synthesised with fruit maturation.

The calcium accumulation pattern followed a sigmoidal model, unlike the other nutrients in the first evaluation months (July, August and September), wherein the fruit in the pinhead stage had an increase in the accumulation of this nutrient (Figure 6). These results agree with those of Laviola et al. (2007c) who found the same behaviour in Arabica coffee, and they link this cause to the importance of this nutrient in cellular division processes and stabilisation of the membranes and cell walls of new cells (Taiz & Zeiger 2010). The calcium accumulation increased until February, the period when the fruit goes through the rapid expansion, bean formation and early maturation stages; however, there was a decrease in the two last months (March and April). Laviola et al. (2007a) also obtained maximum accumulation of this nutrient in these three stages at different cultivation altitudes, and Partelli et al. (2014) obtained maximum calcium accumulation rates in these stages for genotypes of different maturation cycles, observing different behaviours among the genotypes. Nevertheless, both showed an increase until the end of the reproductive cycle.

The magnesium accumulation showed sigmoidal behaviour, and in July to September, there was no increase in its accumulation rate, remaining stable in this period (Figure 7). After October, there was a gradual increase, with a maximum accumulation rate in the last evaluation month. These results agree with those of Partelli et al. (2014) and Laviola et al. (2008), who obtained greater accumulation of this nutrient in the expansion, bean formation and maturation stages, evidencing the high nutritional demand for fruit formation in this reproductive stage in view of the fact that fruits are preferential nutrient drains.

The sulphur accumulation curves behaved differently from those of the other nutrients, and in the initial stage, accumulation was less pronounced until September. An increase in accumulation started after October, but it remained weak until the value doubled in the two last evaluation months (March and April) (Figure 8). Similar results were observed by Laviola et al. (2007d), with greater relative sulphur accumulation in the bean formation/maturation stages in Arabica coffee fruits.

With similar results, Partelli et al. (2014) obtained significant sulphur accumulation in the last evaluation months for genotypes of conilon coffee of early cycle (February), intermediate cycle (March), late cycle (April) and super late cycle (May). Given the above, it is possible to state that mineral fertilisation should be performed as a function of the nutrient accumulation curves in fruits, as they clearly show the stages in which the plant has greater requirements. Thus, in view of the accumulation peaks, it is suggested that fertilisation with macronutrients should be concentrated in higher proportion after October because the fruit is in the rapid expansion stage, extending until bean formation/maturation. However, attention should be paid to the individual behaviour of each nutrient because there are significant differences in accumulation.

Materials and Methods

Location of the experiment

The experiment was conducted in the city of Rolim de Moura, located within the "Zona da Mata" region in the state of Rondonia, Brazil, on a private property established on Line 180, km 11 south, with an average altitude of 277 metres, 11º 49' 43"S latitude and 61º 48' 24"W longitude. The predominant climate in the region is tropical wet - Am (Köppen) with an average annual temperature of 26 ºC and an average rainfall of 2,000 mm year^{-1}. The rainy season is from October-November until April-May. The first quarter of the year has the highest accumulation of rain. The warmest period is between the months of August and October (Cogeo-Sedam, 2012).

Throughout the experiment, the minimum, average and maximum temperature values and rainfall values were collected from the weather station of the Federal University of Rondonia, located in the same city, 23 km from the experimental area. The data are shown in figure 1.

Characteristics of soil and fertilization management

The soil in the region is classified as clayey eutrophic dark red oxisol (Santos et al., 2013), with a flat relief, and its characteristics are shown in table 1. There was a large amount of straw on the ground from the processing of the coffee fruit, which was replaced after the harvest. The fertilisation treatment was performed according to the recommendations for cultivation, at the doses of 440 kg ha^{-1} of nitrogen (N), 270 kg ha^{-1} of potassium chloride (K2O), 9 kg ha^{-1} of phosphorus (P2O5), 12.5 kg ha^{-1} of calcium oxide (CaO) and 6 kg ha^{-1} of sulphur (S). Single superphosphate was applied only once (July 12, 2013) and urea and potassium chloride in four instalments (July 12 and October 22, 2013, January 31 and February 28, 2014). During drought periods, irrigation was conducted by conventional aspersion, and the applied water amount information is shown in Fig 1.

Experiment driving

The study was conducted in a coffee crop propagated via 2.5-year-old cuttings, with spacing of four metres between rows and one metre between plants (2,500 plants per hectare).

The experiment was conducted in a scheme of plots subdivided by time in which the main plots consisted of two fertilisation regimes (fertilised and unfertilised), and the subplots were composed of sampling times of leaves and fruits. The experimental design was of randomised blocks with three repetitions. Each experimental plot consisted of 11 plants, with two plagiotropic branches marked on each plant, with 10 to 12 rosettes in the middle portion of the canopy at the cardinal points north and south.

Samples were collected at 28-day intervals from the pinhead developmental stage (July 2013) until full maturation (April 2014). Five branches from each treatment were randomly collected within the repetitions at each evaluation, completely detaching them from the stem and placing them into paper bags. Twenty-two leaves were collected at the same time from the middle third portion of the plant, after the third pair of leaves, randomly in each block, and the evaluations extended two months after the harvest (June 2014) to verify the behaviour of the leaf nutrient concentrations after fruit extraction.

The collected berries and leaves were placed in a convection oven at 65ºC and dried until constant weight was achieved. Subsequently, separation and counting of the berries was performed, and they were weighed on a precision scale. There were flowers on the branches collected in July, and they were placed with the pinhead berries. The dry material was ground in a stainless steel Wiley mill for the chemical analyses. The analyses were performed using the methodology described by Silva et al. (2009).

Statistical analysis

The accumulation of each nutrient per berry (mg berry^{-1}) was determined by the following formula: Accumulation = dry weight of the berry (grams) x nutrient concentration (grams per kilogram) / 1,000, and the averages were subjected to analysis of variance and regression. The nutrient concentration means in the leaves were only subjected to analysis of variance (ANOVA) using the statistical software Assistat 7.7 beta, and the graphs were prepared from spreadsheets (Silva et al., 2002).

Conclusions

Mineral fertilisation influences the concentration of the nutrients nitrogen and calcium in the fruit and that of nitrogen, phosphorus, potassium and magnesium in leaves.

The fruit and leaf sampling times influence the concentrations of the macronutrients in south western Amazon. The accumulation in fruits differs only for nitrogen, and fertilised plants accumulate higher levels. The other nutrients are not influenced by the fertilisation regime.

The macronutrient accumulation curves behave in a sigmoidal manner, with lower content in the pinhead stage and increased content in the expansion, bean formation and maturation stages, indicating that the sampling time affects the accumulation. However, there are differences among the nutrients. Mineral fertilizer should be concentrated from October to April due to increased demand for nutrients to fruit formation phases of expansion, graining and maturation, but each nutrient must be supplied separately to meet the most demanding stages of the plant.

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