

# Status of Nitrogen in Different Parts of Cupuassu Tree in Oriental Amazon

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#### Abstract

Despite the socio-economic importance of the cupuassu tree for the Amazon region, a few studies have aimed to evaluate the nutritional status of nitrogen in cupuassu plantations, especially in the different vegetative parts. Thus, this research aimed to quantify the content and accumulation of nitrogen in vegetative parts of two progenies of cupuassu trees in the Amazon region. The experiment was carried out in an area of intercropping cupuassu tree x acai palm x banana, at Embrapa Amazônia Oriental, located in Belém, in the state of Pará from 2003 to 2007. The experimental design adopted was completely randomized in a 2 x 4 x 4 factorial scheme with two progenies (PMI 186 (Codajás) and a PMI 215 (Manacapuru)) x four years of evaluation and four plant components (leaves, stems, primary branches and secondary branches) with five replications, in which each experimental unit was composed of a plant from each progeny. The two PMI(s) present nitrogen storage in the secondary branches, but the PMI 186 presented higher N accumulation than the PMI 215. N content was higher in the leaves in both progenies independent of the plant age, with values over 50% of the total content. Therefore, Cupuassu tree progenies under intercropping showed satisfactory N accumulations throughout the study. It can be concluded that it is being viable options in intercropping in agroforestry systems.

Keywords: Agroflorestry, Amazonian soils, mineral nutrition, Theobroma grandiflorum

# 1. Introduction

The cupuassu tree [(*Theobroma grandiflorum* (Willd. ex. Spreng.) Schum)] is a fruit tree species native to the Amazon region. It has a great economic importance for the region, being widely cultivated in agroforestry systems (Gonçalves *et al.*, 2013; Galvão *et al.*, 2017; Silva *et al.*, 2020; Almeida *et al.*, 2021). Among the cupuassu producing states, Pará is one of the biggest producers, producing 27 thousand tons and a yield of 3200 kg ha<sup>-1</sup> (SEDAP, 2021).

The fruit yield and quality result from the interaction of several factors, including adequate mineral nutrition. Maximum production and optimal fruit quality are achieved when the nutritional status of plants is ideal (Silva *et al.*, 2016). In many agricultural situations, this condition is satisfied by annual supply of fertilizers and correction of soil acidity (Bambolim *et al.*, 2015; Abanto-Rodríguez *et al.*, 2018). Among the essential nutrients for the development of cupuassu, nitrogen is the most critical because it is crucial for several physiological processes in the plant. It is a constituent element of amino acids, proteins, and nucleic acids. Plants with low nitrogen contents show reduced growth and yield (Malavolta, 2006; Taiz *et al.*, 2017; Kerbauy, 2019).

Several studies confirmed that fruit trees are strongly responsive to the addition of nitrogen fertilizers, positively reflecting on development and productivity (Amorim *et al.*, 2015; Paramo *et al.*, 2016; Abanto-Rodríguez *et al.*, 2018). However, despite the great importance of cupuassu cultivation in the Amazon, studies related to extracting, exporting, and accumulating nitrogen in the cupuassu tree are scarce and limited to the leaves (Alfaia and Ayres, 2004). Thus, there is a need for studies aiming at the status assessment of nitrogen in different vegetative parts of cupuassu trees in the Amazon, considering that this information



is essential for formulating adequate fertilizers that provide high yields. Thus, the study aimed to quantify the content and accumulation of nitrogen in different vegetative parts of two progenies of cupuassu in the Amazon region.

# 2. Material and Methods

The experiment was carried out in an area of intercropping cupuassu tree x açai palm x banana crop from 2003 to 2007, at Embrapa Amazônia Oriental, located in Belém, in the state of Pará, between the coordinates  $48^{\circ}26'55''$  W -  $48^{\circ}26'40''$  W and  $01^{\circ}26'30''$  S -  $01^{\circ}26'10''$  S. The monthly average air temperature of Belém is 27.1° C and relative air humidity is 80%. According to the Köppen classification, the prevailing climate of the region is Afi-type, with annual average precipitation of 2754.4 mm, with one wet season from December to May and one less wet season from June to November (Bastos *et al.*, 2002). The monthly average of air temperature and precipitation during the study period are shown in Figure 1.



Figure 1. Monthly average of air temperature (°C) and precipitation (mm) during the entire study period

# 2.1 Experimental Arrangement

The experimental area has approximately  $4,300 \text{ m}^2$  with plants of cupuassu progenies 186 and 215 planted and arranged in rows in an alternating manner at a spacing of 5 x 5 m, intercropped with a 2.2 x 2.5 m banana tree, used as temporary shading and açai palm tree as definitive shading (10 x 10 m). In addition, mahogany was planted in the borders (20 x 10 m). The research was carried out for four years, with annual evaluations of 5 plants of each progeny, with 40 plants.

# 2.2 Soil Samplings

The soil of the experimental area was classified as Oxisol (Soil Survey Staff, 2014). The chemical analyzes performed before and during the experiment are shown in Table 1 and 2.

They were determined according to the methodology proposed by Raij et al. (2001).

	deep	pН	Р	K	Ca	Ca+Mg	Al
	( <b>cm</b> )	$H_2O$	mg d	lm <sup>-3</sup>	Cmo		
Sample	0-20	4,3	4	19	0,2	0,4	0,8

Table 1. Chemical properties of the soil in the experimental area before of the study

Table 2. Chemical properties of the soil in the experimental area at the end of the study

Samples	deep	pН	OM	Р	K	Na	Ca	Ca+Mg	Al	H+Al
	(cm)			mg dm <sup>-3</sup>			cmolc dm <sup>-3</sup>			
		H <sub>2</sub> O	g kg							
PMI 186	0-20	4.2	8.36	193	49	16	0.8	1.3	0.9	3.96
PMI 215	0-20	4.0	8.91	206	61	21	1.0	1.7	1.1	5.45

pH = hydrogenation potential; OM = organic matter; P = phosphorus; K = potassium; Na = sodium; Ca = calcium; Mg = magnesium; H = hydrogen; Al = aluminum.

# 2.3 Plant Material Selection

Seeds of 186 and 215 clones were collected to produce the seedling used in the present study. These clones were chosen for presenting traits of high fruit yield and tolerance to witches' broom. The seedlings one-year-old were transplanted. In the third year of the study, flowering began in the rainy season (in April), and fruit production started in September (end of the rainy season). The precocity of the material selected for this research was characterizing; the fruit production interval was different from one progeny to another.

# 2.4 Fertilization

Fertilization in the cupuassu x açaí x banana tree intercropping system was started by the banana trees, one year before implementing the cupuassu tree with 500 g of triple superphosphate (TSP) per plant. Two months later, there was fertilization with 1 kg of castor bean pie (TM) per plant and 200 g of potassium chloride (KCl) per plant in the following month. Three months later, the topdressing fertilization was carried out with 250 g of urea, 250 g of potassium chloride (KCl), 100g of dolomitic limestone as a source of magnesium, applied in top dressing only in the first year of banana and 10 g of FTE (micronutrient) per plant. In the one-year-old açaí palm, topdressing fertilization with 70 g of urea, 100 g of TSP, 85 g of KCl, 90 g of magnesium sulfate, and 5 g of FTE was carried out per plant. In the cupuassu tree in the year of implantation, fertilization was carried out in a hole of 40 cm x 40 cm x 40 cm, with 25 g of TSP, 100 g of KCl, and 500 g of TM.

Two months later, there was a top dressing with 100 g of urea, 200 g of KCl, 50 g of TSP, and 50 g of magnesium sulfate per plant. In the following month, organic fertilization was carried out with 1 kg of TM per plant and covered with grass and banana leaves. In the second year, topdressing fertilization was performed with 107 g of urea, 178 g of TSP, 142 g of KCl, 20 g

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of FTE, and 50 g of magnesium sulfate. In the third year of age, fertilization was carried out with 180 g of urea, 200 g of TSP, 170 g of KCl, 200 g of magnesium sulfate, 10 g of FTE. In the fourth year, the last fertilization was carried out with 200 g of urea, 100 g of KCl, 100 g of TSP, 100 g of magnesium sulfate, and 20 g of FTE per plant.

# 2.5 Collection and Preparation of Plant Samples for Nutrition Analyses

Cupuassu plants were chosen randomly, using the direct destructive method of trees proposed by Corte and Sanquetta (2007) and Carmo *et al.* (2003), using the whole cupuassu tree. Each plant component was collected (leaves, stems, and branches) and placed on plastic canvas. The leaves were collected according to the methodology described by Sodré *et al.* (2001) for the cocoa tree. The process consists of collecting leaves from the apex of a newly matured shoot, with 60 - 90 days, at half-height of the canopy, in the third pair of alternate leaves, avoiding branches with new shoots.

In the set of branches, all the fragments were removed and located in different positions of the tree crown, which were mixed to form a composite sample. From this sample, a subsample of 500 g was taken, which was duly weighed with the aid of an analytical balance for the determination of fresh weight and then conditioned and labeled in a Kraft paper bag and taken to dry in a regulated forced air oven at a temperature of 70 °C, until reaching constant mass weight.

The cupuassu tree stem was weighed whole, and a disk-shaped sample was removed with a chainsaw, as described by Oliveira Neto *et al.* (2003). After drying in the oven, with constant weight obtained, the dry mass of samples from the different components of the cupuassu tree was determined.

# 2.6 Determination of Dry Mass and Nitrogen Contents and Accumulations

The dry mass was determined by weighing the components of the cupuassu plants on an analytical scale and ground in a Willey-type mill with a 2 mm mesh sieve. The ground samples were placed in plastic bags previously identified and divided into two parts. One part was sent to the Soil Laboratory of Embrapa Amazônia Oriental to determine nitrogen contents as recommended by Embrapa (1999). The nitrogen content was determined by the analytical principle of distillation expressed in g kg<sup>-1</sup>. Nitrogen accumulation was determined based on dry mass (g plant<sup>-1</sup>), according to Equation 1, with these results expressed in mg plant<sup>-1</sup>.

N acumulation = 
$$\frac{\text{dry mass (L, S, PB and SB) x N content}}{1000}$$
 (1)

# 2.7 Experimental Design and Statistical Analysis

The experimental design adopted was completely randomized in a  $2 \times 4 \times 4$  factorial scheme with two progenies (PMI 186 (Codajás) and PMI 215 (Manacapuru)) x four years of evaluation and four plant components (leaves (L), stems (S), primary branches (PB) and secondary branches (SB)) with five replications, resulting in 40 experimental units. Each



experimental unit was composed of a plant from each progeny. Regression and variance analyzes were performed for nitrogen within progenies 186 and 215 and plant parts (L, S, PB and SB).

# 3. Results and Discussion

# 3.1 Nitrogen Content in the Progenies

There were not significant differences (p>0.05) of N content in different vegetative parts in progenies 186 and 215, and progenies x years.

# 3.2 Nitrogen Accumulation in the Progenies

The results of nitrogen accumulation in leaves (L), stems (S), primary (RP), and secondary (RS) branches in the two progenies of cupuassu tree (PMI 186 and PMI 215) according to the age presented quadratic behavior (Figure 2). The two progenies accumulate almost the same value of nitrogen in the leaves in the fourth year. The N content in leaves ranged from 6,096.79 mg plant<sup>-1</sup> (second year) to 34,055.41 mg plant<sup>-1</sup> (third year) in the progeny 186 and from 11,890.30 mg plant<sup>-1</sup> (second year) to 28,928.79 mg plant<sup>-1</sup> (third year) in the progeny 215 (Figure 2A). In the progeny 186, the accumulation of N in the stem was from 1,215.39 mg plant<sup>-1</sup> (second year) to 7,322.01 mg plant<sup>-1</sup> (fourth year), and in the progeny 215, it was from 2,005.44 mg plant<sup>-1</sup> (second year) to 10,248.88 mg plant<sup>-1</sup> of N (fourth year) (Figure 2B).

In the primary branches, the progeny 186 showed a greater nitrogen accumulation from the second (1,535.17 mg plant<sup>-1</sup>) to the fourth year (14,397.20 mg plant<sup>-1</sup>). The progeny 215 accumulated 2480.01 mg plant<sup>-1</sup> (in the second year) and 11,886.05 mg plant<sup>-1</sup> of N (in the fourth year) (Figure 2C). The two PMI(s) present nitrogen storage in the secondary branches, but the PMI 186 presented higher N accumulation than the PMI 215 (Figure 2D).

The high nitrogen levels obtained in the third and fourth years of progenies 186 and 215 may be attributed to nitrogen fertilization or the efficiency of these plants in absorbing and translocating the element; thus, the progenies were responsive to nitrogen fertilization (Figure 2). Joshi *et al.* (2016) also observed an increase in N content in the leaves in mango trees under different fertilizations, until the flowering stage in India.







Figure 2. N accumulation in the leaves (A), stem (B), primary (C), and secondary branch (D) for the progenies 186 and 215 according to the age

Nitrogen fertilization is crucial for the full development of plants, considering that N, in addition to its transport function, plays a role in the production of nitrogen compounds in the many processes of ionic absorption, photosynthesis, respiration, multiplication and cell differentiation, and synthesis in general. Besides all these processes, it is part of the structure of amino acids, proteins, nitrogenous bases, nucleic acids, enzymes and coenzymes, vitamins, glucose and lipoproteins, pigments, and secondary products (Amorim *et al.*, 2015; Taiz *et al.*, 2017; Kerbauy, 2019). Nitrogen does not dispense with the participation of other elements, which the absence of the other elements decreases the leaf area index and fruit yield (Malavolta, 2006). Bellote *et al.* (1980), in their studies of nutrient extraction and exportation by Eucalyptus grandis at four and a half years of age, found the highest accumulation of nitrogen in stems with 62 mg plant<sup>-1</sup>, while leaves were with 48.80 mg plant<sup>-1</sup> and branches with 6.39 mg plant<sup>-1</sup>. These accumulations were lower than those found in this research.

Figure 3 shows that in the progeny 186, only in the fourth year, the leaf presented the lowest nitrogen accumulation compared to other years with 56.0% against 25.3% in RS. In the fourth year, the leaf was in first, the secondary branches in second, and the primary branches (RP) in third (12.4%), and finally the stem with 6.3% (Figure 3A).

In the progeny 215, only in the fourth year, the leaf presented lower nitrogen accumulation concerning other years with 56.61% against 24.32% in RS (secondary branch). In all years, the leaf dominated as the first nitrogen store (Figure 3B). In the fourth year, the leaf was in first, the secondary branches in second, the primary branches (RP) in third (10.3%), and finally the stem with 8.8%. The greater demand for nitrogen accumulation in the leaf may be associated with new leaf releases and the absence of pruning for both cupuassu progenies.





Figure 3. Percentage distribution of accumulated nitrogen in different parts of cupuassu plants from PMI 186 (A) and PMI 215 (B)

In this research, it is important to highlight the high accumulation of N in the leaves, representing at least 50% of the total accumulation of N in the vegetative parts evaluated (Figure 3). Laviola and Dias (2008) also observed a more significant accumulation of nitrogen in jatropha leaves. Nurafiza *et al.* (2017) evaluated the N, P, and K contents in different compartments in 10-year-old cocoa intercropped with other crops in Malaysia and observed higher N content in the leaf than in the stem and branches. Furthermore, the authors stated that this response was possibly due to the rapid translocation of N from roots to leaves due to the transpiration process.

# 4. Conclusion

The most significant accumulations of N in the different vegetative parts occurred from the  $3^{rd}$  year of age.

Cupuassu tree progenies in intercropping showed satisfactory N accumulations throughout the study, being viable options in agroforestry systems.



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