FERTILIZATION AND COVER CROP EFFECTS ON SOIL NITROGEN AND PLANT NUTRITION IN A YOUNG GUARANA PLANTATION

Lucerina TRUJILLO[1,2], Johannes LEHMANN[3,*], Manoel da Silva CRAVO[1,4], André Luiz ATROCH[1], Firmino José do NASCIMENTO FILHO[1]

ABSTRACT - Fruit tree production is gaining an increasing importance in the central Amazon and elsewhere in the humid tropics, but very little is known about the nutrient dynamics in the soil-plant system. The present study quantified the effects of fertilization and cover cropping with a legume (*Pueraria phaseoloides* (Roxb.) Benth.) on soil nitrogen (N) dynamics and plant nutrition in a young guarana plantation (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) on a highly weathered Xanthic Ferralsol. Large subsoil nitrate (NO_3^-) accumulation at 0.3-3 m below the guarana plantation indicated N leaching from the topsoil. The NO_3^- contents to a depth of 2 m were 2.4 times greater between the trees than underneath unfertilized trees ($P<0.05$). The legume cover crop between the trees increased soil N availability as shown by elevated aerobic N mineralization and lower N immobilization in microbial biomass. The guarana N nutrition and yield did not benefit from the N input by biological fixation of atmospheric N_2 by the legume cover ($P>0.05$). Even without a legume intercrop, large amounts of NO_3^- were found in the subsoil between unfertilized trees. Subsoil NO_3^- between the trees could be utilized, however, by fertilized guarana. This can be explained by a more vigorous growth of fertilized trees which had a larger nutrient demand and exploited a larger soil volume. With a legume cover crop, however, more mineral N was available at the topsoil which was leached into the subsoil and consequently accumulated at 0.3-3 m depth. Fertilizer additions of P and K were needed to increase subsoil NO_3^- use between trees.

Key-words: acid tropical soil, cover crop, Brazilian Amazon, fruit tree, nitrogen leaching

Efeitos da fertilização mineral e da cobertura do solo sobre a dinâmica do Nitrogênio e na nutrição de plantas jovens de guaraná

RESUMO - A produção de fruteiras está ganhando grande importância na Amazônia Central e em outras partes dos trópicos úmidos mas, muito pouco ainda é conhecido sobre a dinâmica de nutrientes no sistema solo-planta. O presente estudo quantificou os efeitos da fertilização mineral e da cobertura do solo com uma leguminosa (*Pueraria phaseoloides (Roxb) Benth.)* sobre a dinâmica do N no solo e sobre a nutrição de plantas jovens de guaraná (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke), em um Latossolo Amarelo muito argiloso. Grande acúmulo de nitrato (NO_3-) encontrado na profundidade de 0,3 – 3,0m abaixo do plantio de guaraná é um indicativo da lixiviação de N da camada superficial. Os teores de NO_3- na profundidade de 2m era 2,4 vezes maior entre as plantas do que na entrelinha que não recebeu fertilização ($P<0,05$). A leguminosa de cobertura, entre as plantas de guaraná, aumentou a disponibilidade de N, conforme

[1]Empresa Brasileira de Pesquisa Agropecuaria (Embrapa)- Amazônia Ocidental, Caixa Postal 319, 69011-970 Manaus, Brazil

[2]present address: Instituto Nacional de Pesquisa da Amazonia (INPA), Manaus-Amazonas, Brazil

[3]Institute of Soil Science and Soil Geography, University of Bayreuth, Universitätsstr. 30, D-95440 Bayreuth, Germany

[4]present address: Empresa Brasileira de Pesquisa Agropecuaria (Embrapa) - Amazônia Oriental, Belem-Para, Brazil

*Corresponding author, fax: 001-607-255-8615; email: CL273 @cornell.edu; Department of Crop and Soil Sciences, Cornell University, Ithaca, NY 14853, USA

é indicado pela elevada mineralização aeróbica e baixa imobilização de N na massa microbiana. A nutrição nitrogenada e a produção do guaraná não foram beneficiados pela adição de N, via fixação biológica do N_2 da atmosfera pela leguminosa de cobertura (P<0,05). Mesmo sem leguminosa nas entrelinhas de plantio, grandes quantidades de NO_3- foram encontradas no subsolo, entre plantas não adubadas. O NO_3- do subsolo entre as plantas pode, entretanto, ter sido utilizado pelo guaraná fertilizado. Isso pode ser explicado pelo crescimento mais vigoroso das plantas fertilizadas as quais têm uma grande demanda por nutrientes e exploraram maior volume de solo. Com uma leguminosa de cobertura, contudo, mais N mineral foi disponibilizado na camada superficial, o qual foi lixiviado para o subsolo e, consequentemente, acumulado na camada de 0,3 a 3,0m de profundidade. Adições suplementares de P e K foram necessárias para aumentar a utilização do NO_3- entre as plantas.

Palavras-chave: Amazônia brasileira, Solos tropicais ácidos, Plantas de Cobertura, Fruteiras, Lixiviação de Nitrogênio.

INTRODUCTION

Soil fertility is generally very low in the highly weathered soils of the central Amazon and sound nutrient management practices are essential for continuous crop production (Cravo and Smyth, 1997). Indigenous fruit trees are an important local commodity in the Amazon and other humid tropical regions but also receive increasing attention as cash crops for domestic and international markets. Little is known, however, about the nutrient productivity and the uptake of N by these tree crops. The high rainfall intensity and the high hydraulic conductivity of the central Amazonian upland soils contribute to large and rapid water percolation (Rozanski *et al.*, 1991) and, therefore, also to N leaching (Melgar *et al.*, 1992; Cahn *et al.*, 1993). At the same time, the subsoil contains large amounts of variable charge clay minerals and was reported to have high anion exchange capacity and NO_3^- sorption capacity increasing to a depth of 1.2 m (Cahn *et al.*, 1992). As a result, large NO_3^- accumulations were observed under several fertilized fruit trees on an acid upland soil in the central Amazon (Schroth *et al.*, 1999). Nitrogen leaching measured by nitrate profiles was highest between the rows of oil palms in the central Amazon (Schroth *et al.*, 2000). The NO_3^- profiles allowed an assessment of N losses from the topsoil and revealed a valuable source of N which could potentially be recycled by deep rooting trees.

After forest clearing, the soils in the humid tropical environment are susceptible to erosion unless they are protected by a vegetation cover. Introducing cover crops into tree plantations is a wide-spread technique to prevent soil degradation or even improve soil fertility. In Asia, oil palm and rubber plantations were established with various legume covers for a long time (Broughton, 1977; Agamuthu and Broughton, 1985). Additionally, legume cover crops are able to provide external N sources by biological fixation of atmospheric N_2, which can amount to 150 kg N ha^{-1} yr^{-1} in tree plantations (Giller, 2001). It is unclear whether or not these large amounts of N fixed by the cover legumes can be used by the tree. The N uptake by two Amazonian fruit trees from the topsoil under a legume cover, which was enriched in N, was too low to substantially improve their N nutrition of the trees (Lehmann *et al.*, 2000a). A large proportion of soil N derived from biologically N_2 fixation and mineralization of native soil N may be leached (Melgar *et al.*, 1992; Cahn *et al.*, 1993) and may lead to increased NO_3^- adsorbed to the acid subsoils (Schroth *et al.*, 1999). No information exists if the trees could exploit the subsoil NO_3^- underneath the cover crop.

The cover crops also take up nutrients such as P or K from the soil and may compete for the same resources as the tree crop. This may reduce both, nutrition and production of the trees (Dominguez and Cruz, 1993; Perez *et al.*, 1993; Lehmann *et al.*, 2000b). Little is known about how fertilization affects competition for nutrients other than N between trees and cover crops.

The objectives of the present study were to investigate the effects of fertilization and cover cropping with a legume on spatial and temporal dynamics of soil N distribution, on growth and nutrition of a young guarana (*Paullinia cupana*) plantation under the humid tropical conditions of the central Amazon.

MATERIAL AND METHODS

Study site and experimental setup

The study was carried out in the humid rainforest region of the central Amazon at the Embrapa experimental station 30 km north of Manaus, Brazil. The rainfall distribution is monomodal with a maximum between December and May and a mean annual total of 2503 mm (1971-1993). Mean annual air temperature is 26 °C and atmospheric humidity 85%. The soils are classified as Xanthic Ferralsol (FAO, 1990). They are deep and clayey, with pH (H_2O) of about 4.5, an organic C content of 18.9 mg g^{-1} and a total N content of 1.6 mg g^{1} (0-0.1 m). Topsoils (0-0.1 m) have low P contents of 6.3 mg kg^{-1} and cation exchange capacity of 21 $mmol_c$ kg^{-1} (under fallow vegetation; Schroth *et al.*, 1999). Positive charge ranges from 26 $mmol_c$ kg^{-1} in the topsoil to 48 $mmol_c$ kg^{-1} in the subsoil and does not decrease to a depth of 8 m in a nearby secondary forest (unpublished data).

In 1995, the site was cleared from 10-years-old secondary forest after rubber, the residues were burned and guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke; Sapindaceae) was planted in a grid of 5 m ´ 5 m (400 plants ha^{-1}). The experiment was originally designed for comparing different guarana clones. Guarana was chosen for the present study as it is one of the most important cash crops of the Amazon and almost no information is available about its productivity. Guarana fruits are used for juice and soft drink production. The individual plots consisted of three plants in one row. Around each plot, at least one plot was left with the same treatment as a border. Sufficient distance between treatments was possible since 16 plots were available of the same treatment. The plots were arranged in a randomized complete block design with four replicates using clones as the blocking factor. The present study was conducted four years after planting when the trees went into fruit production. Two main factors were investigated in a full factorial design: fertilization and association with *Pueraria phaseoloides* (Roxb.) Benth. Fabaceae as a cover crop (Table 1). *Pueraria* developed from a seed bank of a previous rubber plantation and was controlled by periodic cutting to avoid infestation of the trees. The biomass production of *Pueraria* was not measured on the presented experimental plots but on nearby fields with similar abundance and determined at 5.1 Mg dry matter ha^{-1} yr^{-1} (Uguen *et al.*, unpublished in Lehmann *et al.*, 2000b). In the plots without legume cover *Pueraria* was manually controlled and had a cover of spontaneous perennial grasses and herbacous plants (*Rolandra fructicosa*, several Ciperaceae and Melastomataceae).

Fertilizer was applied under the tree canopy according to local recommendation (Embrapa, 1998). Annually, each tree received 103.5 g N as urea, 44 g phosphorus (P) and 26 g calcium (Ca) as triple superphosphate, 123 g potassium (K) as KCl, 32.3 g magnesium (Mg) as $MgSO_4$, 4 g zinc (Zn) as $ZnSO_4$, and 1.1 g boron (B) as borax, corresponding to 41.4, 17.6, 10.4, 49.2, 12.9, 1.6, 0.4 kg ha^{-1} yr^{-1}, respectively. The fertilizer was applied close to the stem (app. 1 m radius) at three times a year in January/March/May with 35/30/35, 100/0/0, 0/40/60, 0/50/50, 0/50/50, and 0/50/50 % of the total annual application for urea, triple superphosphate, KCl, $MgSO_4$, $ZnSO_4$, and borax, respectively. At tree planting, additional 5 L chicken manure were applied to each tree with a total of 152 g N, 102 g P and 78 g K. The ^{15}N content ($\delta^{15}N$ of -0.7 ‰) of the applied urea fertilizer was lower as compared to the soil ($\delta^{15}N$ of 8-11 ‰) and, therefore, $\delta^{15}N$ values could be used to trace the fate of the fertilizer N. Similarly, we assessed the effect of the legume N ($\delta^{15}N$ of 1.0-2.8 ‰) on soil and tree N. The isotopic composition of the applied chicken manure could not be analyzed, but reference material from later collections of chicken manure indicated that their $\delta^{15}N$ values were only slightly lower than soil.

Plant and soil sampling

The fresh weight of the guarana seeds was determined in consecutive collections according to normal harvest an reporting procedures from December 1998 to March 1999. Additionally, the maximum elongation of the vine was measured in June 1999. At the end of the rainy season, 15-30 June 1999, and at the end of the dry season, 1-15 December 1999, soil samples were taken at 0.5 m distance from the trees and in the middle between tree rows. One composite sample was obtained from three individual trees in each replicate. For the soil obtained between trees, only positions within the plot were sampled. The soil was sampled with a 0.1-m-root auger at 0-0.1 m depth, with a 0.08-m-diameter Edelmann auger at 0.1-0.3, 0.3-0.5, 0.5-0.8, 0.8-1.2, 1.2-2 m depth, and with a 0.04-m-diameter motor auger (Pionjär 120, Atlas Copco, Germany) at 2-3 m depth. The soil was immediately transported to the laboratory in a cooling box and extracted field-moist within a maximum of two hours as described below.

Soil and plant analyses

Soil mineral N was extracted from 40 g soil with 150 mL 1N KCl for two hours using a horizontal shaker (100 rpm). The supernatant was transferred after 16 hours when the soil had settled. Subsequently, NO_3^- and ammonium (NH_4^+) were measured colorimetrically with a continuous-flow analyzer (Scan Plus Analyzer, Skalar Analytical B.V., Breda, The Netherlands). Soil N mineralization was determined on a subsample from 0-0.1 m depth using 100 g soil incubated at 25 °C and field capacity for 35 days. After the incubation, soil mineral N was extracted as described above. Nitrogen mineralization was calculated from the difference between the total soil mineral N before and after the incubation. Due to an accidental loss of sample material, N mineralization at the end of the dry season could only be determined in the unfertilized treatments without *Pueraria*. In both seasons, N in the microbial biomass from 0-0.1 m depth was determined by the fumigation extraction method according to Brookes *et al*. (1985). Subsamples were fumigated for 24 hours using chloroform. Thirty grams of fumigated and non-fumigated soil were extracted with 100 mL 0.5 M K_2SO_4. Total N in the extract was analyzed after digestion by distillation against an indicator in H_3BO_3 (Tecnal TE 036/1, Sao Paulo, Brazil). The difference between the N contents of fumigated and non-fumigated soils were expressed as microbial biomass N. The water contents of all soils were determined by drying the samples at 105°C for 48 hours. All extractions were done in duplicate.

In December 1999, the youngest fully developed leaves were sampled from guarana. Three leaves were taken from each of the three trees in one replicate plot and combined to one composite sample. The leaves were gently rinsed with deionized water and were dried at 70 °C for 48 hours.

Soil samples from 0-0.3 m and 2-3 m depth were pooled and air-dried. Both soil and plant samples were finely ground with a ball mill (Retsch). Foliar P, K, Ca and Mg were analyzed after wet digestion. Phosphorus was measured colorimetrically with the molybdenum blue method (Olsen and Sommers, 1982), the cations with atomic absorption spectrometry. The natural ^{15}N abundance was measured to estimate the proportion of fertilizer and biologically fixed N in plant and soil. ^{15}N natural abundance and total N contents of leaf and soil samples were determined with an elemental analyzer (Fisons 1108) coupled via a ConFlo II Interface to a Delta S isotope mass spectrometer (FINNIGAN MAT, San Jose, CA).

Statistical analyses

The analyses of variance were computed using a split-plot design (STATISTICA Version 5, StatSoft, Inc., Tulsa, OK) with the main factors fertilization and cover cropping and the subplot factor position (under the canopy or between trees). In case of significant main or subplot effects or interactions, individual cell means for the respective level were compared using least significant differences (LSD) according to Little and Hills (1978).

RESULTS

Growth, yield and foliar nutrient contents

Fertilized guarana with *Pueraria* had larger maximum vine length than unfertilized trees with *Pueraria*. Without ground cover, fertilization of guarana had no effect on maximum vine length (Table 1). Fertilization and intercropping with *Pueraria* significantly increased fruit yield as compared to guarana which was not fertilized and not intercropped with *Pueraria* ($P<0.05$). Tree growth and crop yields did not change, however, in association with *Pueraria*. The highest foliar N contents were observed in guarana trees which received fertilizer and which were intercropped with *Pueraria* as well as those which did not receive fertilizer and were not intercropped with *Pueraria*. This apparent contrast can be explained by the very poor plant growth of the unfertilized guarana (Table 1) and a higher concentration of N in a lower biomass. In contrast, P nutrition improved when guarana was not associated with a cover crop (significant main effect of cover cropping; $P<0.05$). Foliar Mg and K contents were higher in fertilized guarana with *Pueraria* than unfertilized trees without a cover crop, whereas it was opposite for Ca.

Table 1. Growth, fresh seed yield and foliar nutrient contencentrations of guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) as affected by fertilization and association with a legume cover crop (*Pueraria phaseoloides* (Roxb.) Benth.) in the central Amazon at the end of the dry season. Values in one column followed by the same letter are not signficantly different at $P<0.05$; $n=4$.

Treatment	Maximum vine length (m)	Seed yield (kg tree^{-1})	N	P	K	Ca	Mg	$\delta^{15}N$[1]
			(mg g^{-1} DM)					(‰)
Fertilization with *Pueraria*	5.3 a	4.5 a	33.8 a	3.74 ab	10.2 a	3.99 b	1.37 a	4.82 a
Fertilization without *Pueraria*	5.6 a	3.2 ab	31.0 b	4.11 a	9.5 a	5.35 ab	1.29 ab	5.00 a
No fertilization with *Pueraria*	2.7 b	1.4 ab	30.6 b	3.25 b	9.5 a	5.38 a	1.14 ab	5.64 a
No fertilization without *Pueraria*	4.2 ab	0.7 b	33.9 a	4.16 a	7.3 b	5.60 a	1.03 b	5.77 a

[1] Urea fertilizer had a $\delta^{15}N$ value of –0.7 ‰; *Pueraria* at a nearby site was reported to have $\delta^{15}N$ values of 1.0-2.8 ‰ (Lehmann et al., 2001b)

Soil mineral N contents

The soil NH_4^+ and total N contents significantly ($P<0.05$) decreased below 0.1 m depth without any differences between treatments ($P>0.05$) and were therefore not discussed further (data not shown). The effects of fertilization and cover crop were only expressed in soil NO_3^- contents. During the rainy season, topsoil NO_3^- contents were not affected by fertilization or by cover cropping with *Pueraria* (Figure 1). In the subsoil, however, NO_3^- contents were higher between the trees than under the trees (0.3-0.5 m $P<0.05$; 0.5-0.8 m $P<0.01$; 0.8-1.2 m $P<0.05$) apart from the fertilized guarana without the *Pueraria* cover crop ($P>0.05$). Additionally, soil NO_3^- contents at 1.2-2 m depth under the trees were elevated in fertilized guarana compared to unfertilized guarana without the cover crop ($P<0.05$).

At the end of the dry season, subsoil NO_3^- contents were significantly ($P<0.05$) lower than at the end of the rainy season (Figure 2). In the topsoil, however, NO_3^- contents increased under *Pueraria* ($P<0.05$). To a depth of 2 m, the presence of the legume cover crop significantly increased soil NO_3^- contents between guarana trees 2.3 and 2.5 times in the dry and rainy season, respectively, compared to plots without the legume cover ($P<0.05$). At the end of the dry season this effect was more pronounced down to a depth of 0.5 m ($P<0.06$) than between 0.5, 0.8 and 1.2 m ($P=0.06$ and 0.08, respectively), and not visible below 1.2 m ($P>0.1$).

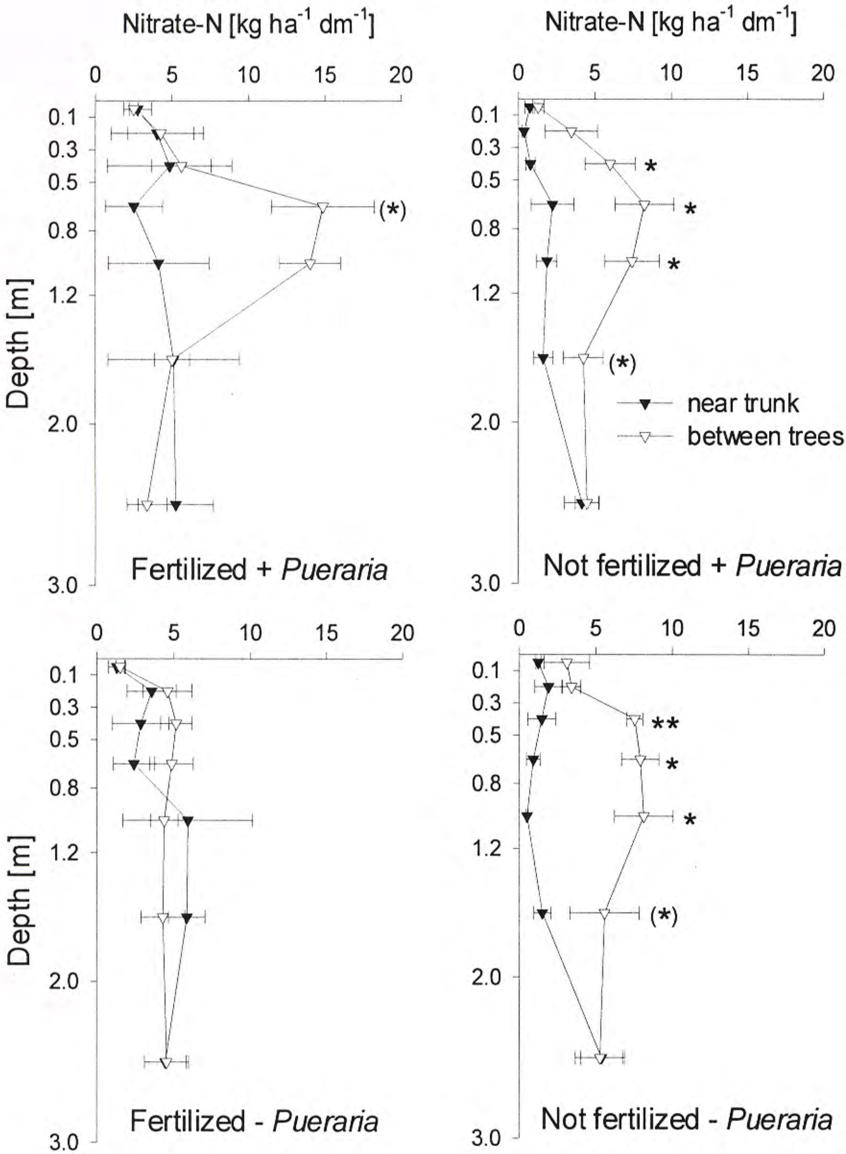

Figure 1 - Soil NO_3^- contents under guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) as affected by fertilization and association with a legume cover crop (*Pueraria phaseoloides* (Roxb.) Benth.) in the central Amazon at the end of the rainy season. (*), *, ** at the error bars indicate significant differences between the mean values under the tree canopy and the alley at $P < 0.1$, 0.05, and 0.01, respectively; absence of asterics indicate non significant differences (n=4).

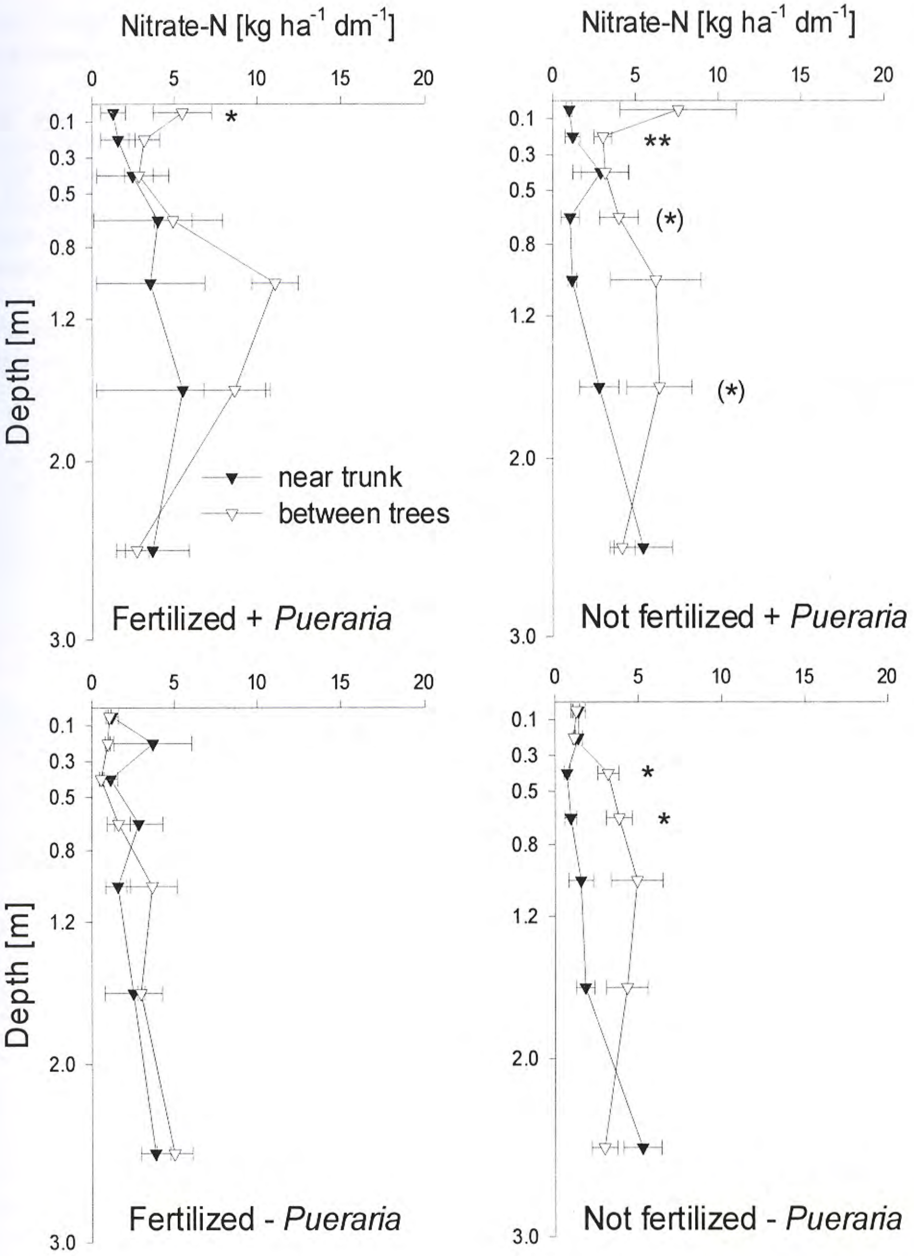

Figure 2 - Soil NO_3^- contents under guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) as affected by fertilization and association with a legume cover crop (*Pueraria phaseoloides* (Roxb.) Benth.) in the central Amazon at the end of the dry season. (*), *, ** at the error bars indicate significant differences between the mean values under the tree canopy and the alley at $P < 0.1$, 0.05, and 0.01, respectively; absence of asterics indicate not significant differences (n=4).

Nitrogen mineralization and microbial biomass N

Nitrogen mineralization in the topsoil (0-0.1 m) collected at the end of the rainy season was higher under the tree canopy than between the trees when the trees were fertilized, but lower when they were not fertilized for pooled cover crop effects (significant interaction position×fertilization $P<0.05$; Table 2). Both, fertilization and cover cropping with *Pueraria* increased soil N mineralization. Fertilization was more effective in that respect ($P=0.03$) than the legume cover crop ($P=0.09$). Nitrogen mineralization significantly ($P<0.05$) increased during the dry in comparison to the rainy season.

In contrast, the N contents in the microbial biomass did not significantly change between dry and rainy season (Table 2). During the dry season microbial N was slightly higher under the tree canopies than between trees ($P<0.1$), but not during the rainy season ($P=0.49$). The presence of the legume cover crop significantly ($P<0.05$) decreased the amount of microbial N. Fertilization had no effect, however, on microbial N contents in soil ($P<0.05$).

Table 2 - Nitrogen mineralization (35 days) and microbial biomass N in the topsoil (0-0.1 m) under and between guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) as affected by fertilization and association with a legume cover crop (*Pueraria phaseoloides* (Roxb.) Benth.) in the central Amazon at the end of the dry season and rainy season (n=4).

Treatment (main effects)	Position (subplots)	N mineralization (kg ha^{-1} dm^{-1})		Microbial biomass N (µg g^{-1} soil)	
		rainy season	dry season	rainy season	dry season
Fertilization with *Pueraria*	near trunk	43.2	n.d.	29.7	35.4
Fertilization with *Pueraria*	between trees	34.7	n.d.	24.8	28.2
Fertilization without *Pueraria*	near trunk	31.5	n.d.	40.7	52.8
Fertilization without *Pueraria*	between trees	28.9	n.d.	43.6	36.7
No fertilization with *Pueraria*	near trunk	21.4	n.d.	25.6	35.5
No fertilization with *Pueraria*	between trees	28.4	n.d.	30.5	29.4
No fertilization without *Pueraria*	near trunk	15.6	25.1	34.5	42.0
No fertilization without *Pueraria*	between trees	20.4	29.5	40.1	39.4
LSD (0.05) between main effects		16.8	n.d.	8.6	22.7
LSD (0.05) within main effects between subplots		13.4	26.3	11.6	15.2

n.d. not determined

Nitrogen-15 in plant and soil

Both the applied urea fertilizer and the *Pueraria* biomass had lower ^{15}N enrichment with $\delta^{15}N$ values of –0.7 ‰ (this study) and 1.0-2.8 ‰ (Lehmann *et al.*, 2000a), respectively, than soil (8-11 ‰). Consequently, a decrease of $\delta^{15}N$ values in soil or plants indicate that either mineral fertilizer or biologically fixed N_2 were the source of N input and diluted soil N which had higher $\delta^{15}N$ values. While the foliar $\delta^{15}N$ values slightly decreased upon fertilization ($P<0.1$), only a marginal effect of the legume was detectable on foliar ^{15}N contents in the trees ($P=0.88$; Table 1). Fertilization and the presence of the legume cover crop decreased the $\delta^{15}N$ values of the topsoil (0-0.1 m; $P<0.05$). Only underneath the fertilized trees, the subsoil showed lower $\delta^{15}N$ values by about 2 ‰ (Figure 3).

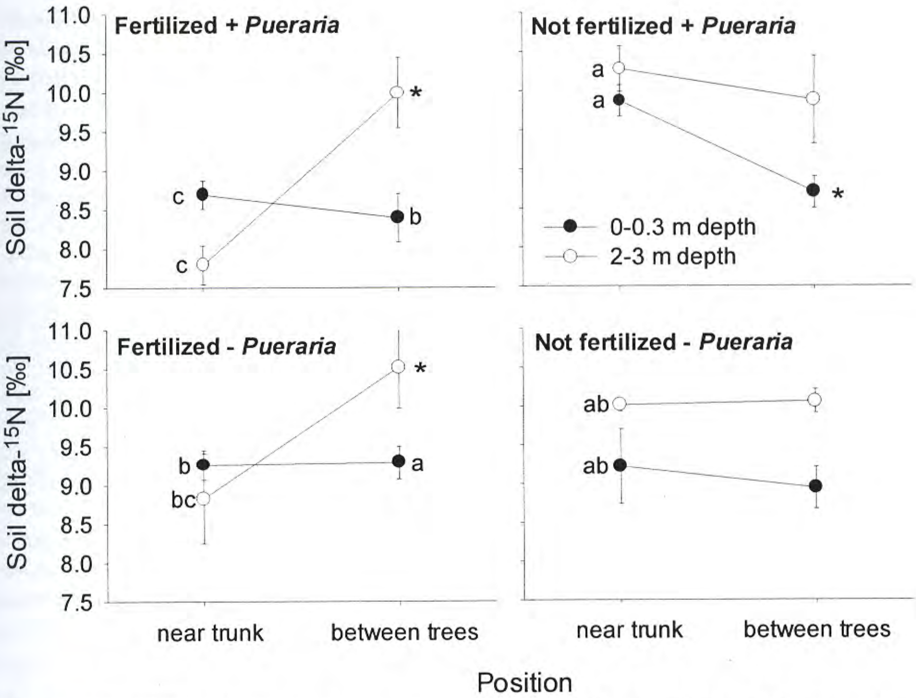

Figure 3 - Soil $\delta^{15}N$ values under guarana (*Paullinia cupana* Kunth. (H.B. and K.) var. *sorbilis* (Mart.) Ducke) as affected by fertilization and association with a legume cover crop (*Pueraria phaseoloides* (Roxb.) Benth.) in the central Amazon at the end of the rainy season. Asterics (*) indicate significant differences ($P < 0.05$) between $\delta^{15}N$ values under the tree canopy and the alley at the same depth; letters indicate significant differences between treatments at the same depth ($P > 0.05$); absence of asterics or letters indicate non significant differences (n=4).

DISCUSSION

Fertilizer and cover crop effects on tree nutrition

Fertilization significantly increased growth and production of guarana. However, it is less obvious which nutrient additions improved guarana nutrition and growth the most. Phosphorus applications did not improve foliar P contents, although the soils are especially deficient in P (Cravo and Smyth, 1997). Magnesium contents increased after fertilization, which is in accordance with critical foliar Mg levels of guarana determined by Castro (1975).

The legume cover crop was able to improve the N nutrition of the fertilized trees. This has been reported from other tree cropping systems. For example, N nutrition and growth of rubber (*Hevea brasiliensis* (Willd.) Muell. Arg.) increased with a legume cover in Malaysia (Broughton, 1977). In Cameroon, coffee (*Coffea arabica* L.) showed higher foliar N contents when intercropped with legumes than with non-legumes or without a cover crop (Bouharmont, 1978). The effects of the cover crop on the N nutrition of the trees depended on the legume species as shown for peachpalm (*Bactris gasipaes* Kunth.) plantations grown with several different cover crops on a Paleudult in Yurimaguas, Peru (Perez *et al.*, 1993). The $\delta^{15}N$ signatures of the guarana at our site indicated, however, that less of the N taken up by guarana was derived from the legume in comparison to the fertilizer. The cover crop may have increased soil N availability due to rapid

N cycling as seen from the elevated N mineralization rather than due to a large input of biologically fixed N_2. A large annual N turnover for *Pueraria* was also calculated in a *Bactris gasipaes* and *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. plantation with 66 % of its total N turnover (Lehmann *et al.*, 2000b).

Without fertilization, however, the N nutrition of guarana decreased due to the presence of the cover crop. The trees were very small and the competition between the cover crop and guarana may have decreased N nutrition of the trees. Also Canto (1989) found reduced foliar N contents of 2-year-old guarana without N fertilization when associated with several different legume cover crops in the central Amazon. Among several nutrients, the deficiency of N was shown to decrease guarana growth the most in a solution experiment by Chepote *et al.* (1983). The legume *Arachis pintoi* (Krap. and Greg.) was also reported to decrease N nutrition and growth of peachpalm on a Humitropept in Turrialba, Costa Rica (Dominguez and Cruz, 1993).

Foliar P contents of guarana significantly decreased due to the association with *Pueraria*. If *Pueraria* is used as a cover crop, additional P may have to be applied to guarana in order to avoid P deficiency in trees. This did not seem to decrease crop production in our study but may be critical after further cropping cycles. A competition for other nutrients was not observed. In fertilized trees foliar Ca decreased compared to unfertilized ones possibly due to antagonisms. Calcium did not seem to limit guarana growth at our site. Calcium may not be as important as other nutrients for guarana growth. Thus, Chepote *et al.* (1983) could show that a fertilization with all essential nutrients but without Ca decreased plant biomass less than the omission of either N, P, K, or Mg in a solution culture. Consequently, foliar Ca contents at our site were still classified as sufficient according to Castro (1975).

Subsoil nitrogen dynamics

A large proportion of the total N in the subsoil under the fertilized guarana derived from the applied urea as shown by the low $\delta^{15}N$ values at 2-3 m depth despite the fact that N was applied in three splits throughout the year. The increase of the subsoil accumulation of NO_3^- at the end of the rainy season ($P<0.05$ over all treatments) demonstrated the effects of high precipitation rates (Nortcliff and Thornes, 1981) and N applications during the wet season. However, the total amounts of adsorbed NO_3^- were still low underneath the trees. The 4-year-old guarana plants were probably able to retrieve nutrients from the subsoil at 0.5-3 m depth as seen from the NO_3^- depletion below the trees. In 5-year-old guarana in Bahia, Brazil, Ramos and Sacramento (1986) still found 9.4 % of the total root mass from 0-1.2 m in 0.9-1.2 m depth (only roots >2 mm diameter studied). The amount of roots increased in 7-year-old guarana, but the depth distribution remained the same.

Larger amounts of mineral N accumulated in the subsoil between the trees, where no fertilizer was applied, than close to the trees. It can not be concluded from these data, whether more N was leached between the trees or the guarana trees took up more N from the subsoil underneath the trunk. However, the existence of large amounts of mineral N in the subsoil indicates that N was leached from the topsoil since no other sources of mineral N are possible. The clear decrease in $\delta^{15}N$ values in the topsoil underneath the *Pueraria* indicates a large contribution of biologically fixed N_2 to soil N. Similarly, Lehmann *et al.* (2001) found higher N contents in labile organic matter fractions indicating greater N availability under *Pueraria* than under four indigenous fruit trees at a nearby site.

Although soil mineral N contents were higher under the legume cover crop and less N was immobilized in microbial biomass, lower amounts of N from biological N_2 fixation were found in the subsoil between guarana than fertilizer N in the subsoil underneath the trees. This was indicated by the more pronounced $\delta^{15}N$ decrease in subsoils underneath the trees than the legume cover between the trees. The greater accumulation of subsoil NO_3^- between the trees than underneath them was rather a result of low N uptake than of high N leaching from the topsoil, because N mineralization was lower between the trees than near the trunk of fertilized guarana. In unfertilized guarana, the situation was reverse. More mineral N was found in the subsoil underneath the legume than the guarana. Therefore a better N utilization from the area between the trees is important to decrease unproductive N losses.

Utilization of soil N as affected by fertilization

Unfertilized guarana did not take up efficiently the N mineralized in the topsoil, because NO_3^- accumulated in the subsoil at 0.5-2 m depth. Similarly, Melgar *et al.* (1992) reported large NO_3^- losses by leaching from urea applied to maize, and Schroth *et al.* (1999) found large accumulations of NO_3^- in the subsoil under four tree crops at a nearby site. In our study the depletion of subsoil NO_3^- and during the dry season even topsoil NO_3^- was lowest between the trees. The accumulation of subsoil NO_3^- between the trees only decreased when guarana received fertilizer and no *Pueraria* cover crop was present. The root system and therefore nutrient uptake may have been insufficient between the trees to absorb all NO_3^- that was biologically fixed by the legume. If the trees were not fertilized, they may not be able to utilize the NO_3^- which was derived from soil mineralization alone. Therefore, guarana may need fertilizer inputs other than N (especially P, K, and Mg) to produce an extensive root system to utilize subsoil nutrients also between trees.

Subsoil nutrient uptake is particularly important in the studied humid tropical environment, where high precipitation and high hydraulic conductivity of soils (up to 1.6 m h^{-1} in 0.15-0.6 m depth in comparable soils; Nortcliff and Thornes, 1981) pose large risks for nutrient leaching. An effective uptake of mobile nutrients from the topsoil such as NO_3^- may not be possible under the high leaching conditions in central Amazonian Ferralsols. NO_3^- was leached from the topsoil even under nearby primary forest trees (Schroth *et al.*, 1999).

Fertilizer and cover crop management

The assessment of NO_3^- profiles were useful to demonstrate N accumulation in the subsoil and its potential availability for deeper rooting trees. Without fertilization, guarana was not able to utilize subsoil N derived from N mineralization between the trees. Lower N applications at similar levels of P, K, Mg, and Ca may stimulate the uptake of N between guarana stands. Care has to be taken to maintain optimal N nutrition, however, as N is the most important nutrient for guarana growth (Chepote *et al.*, 1983). A higher N uptake by guarana from soil covered with *Pueraria* may even increase the amount of atmospheric N fixed by the legume. Similarly, Jayasundara *et al.* (1997) could show that planting *Gliricidia sepium* and *Leucaena leucocephala* in association with grass increased their biological N fixation by 6-21 % on a Ferralsol in Sri Lanka. The competition for other nutrients, however, must be corrected with fertilizer applications, such as P. The P additions used in the present experiment were not sufficient to eliminate competition between cover crop and tree.

Fertilization (especially with P, K and Mg) effectively decreased the accumulation of mineralized N in the subsoil between the trees. Additional N input by biological N fixation in the legume cover crop could not be entirely utilized by the trees. Lower N fertilizer applications may decrease unproductive fertilizer N losses underneath the trees, but may also improve the utilization of mineralized N and the biologically fixed N_2 supplied by the legume cover crop. Fertilizer management must consider the spatial and temporal distribution of N availability in tree cropping systems. The effects of broadcast fertilizer placements with low N on subsoil NO_3^- use warrant further research to achieve a more efficient utilization of available N.

CONCLUSIONS

The guarana N nutrition and yield did not benefit from the N input by biological fixation of atmospheric N_2 by the legume cover.

Even without a legume intercrop, large amounts of NO_3^- were found in the subsoil between unfertilized trees. Subsoil NO_3^- between the trees could be utilized, however, by fertilized guarana. This can be explained by a more vigorous growth of fertilized trees which had a larger nutrient demand and exploited a larger soil volume. With a legume cover crop, however, more mineral N was available at the topsoil which was leached into the subsoil and consequently accumulated at 0.3-3 m depth. Fertilizer additions of P and K are necessary to increase subsoil NO_3^- use between trees. Future research is warranted to increase subsoil N use by guarana and to improve the utilization of biologically fixed N_2 of an intercropped legume by guarana.

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