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Food quantity and quality of cassava affected by leguminous residues and inorganic nitrogen application in a soil of low natural fertility of the humid tropics

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ABSTRACT: The aim of this study was to test the hypothesis that the quality and quantity of biofortified cassava root in a humid tropical environment can be modified with the application of a combination of low- and high-quality residues of leguminous tree species. The experiment was designed as a 6 × 2 factorial (a combination of 4 legume species *versus* 2 levels of nitrogen) with 4 replications in a randomized block design and the following treatments: *Gliricidia* + *Acacia*, *Gliricidia* + *Leucaena*, *Gliricidia* + *Clitoria*, *Leucaena* + *Acacia*, *Leucaena* + *Clitoria*, and a control without legumes. We analyzed the shoot weight, number of roots/plant, root weight, root production, proximate composition, as well as the mineral, carotenoid, and pro-vitamin A contents. Root production increased with the application of high-quality residues. The protein level influenced the carotenoid content. The allelopathic effect of exotic genera — *Leucaena* and *Acacia* —, especially when combined, can decrease the mineral content such as potassium and, therefore, reduce the accumulation of starch.

Key words: biofortification, food security, green manure, nutrients.

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INTRODUCTION

Cassava is one of the first crops that were domesticated and has been used for centuries as a food source by many cultures. This crop is consumed by millions of people in tropical and subtropical regions, being a main food staple in large regions of Africa and Latin America. Cassava is vital for both food security and income generation for these people and their families (Kaweewong et al. 2013). The important role cassava plays in maintaining food security can be attributed to its ease of cultivation and tolerance of poor soils, low rainfall, and high temperatures. In addition, cassava does not require management as intensive as that required by other crops, such as maize, wheat, and rice; besides, it can withstand drought and be stored in fields for long periods of time (Burns et al. 2012).

A meal comprising 500 g of cassava products provides almost the entire minimum daily requirement for energy needs, but only a small part of the minimum daily requirement of vitamin A, protein, iron, and zinc. This is especially critical for poor families for whom cassava constitutes almost the entire daily primary meal and who cannot afford the expensive alternative sources of these nutrients (Montagnac et al. 2009). This has resulted in the need to develop an alternative that provides these essential nutrients as well as the energy requirements of the body.

Therefore, while product quantity is the principal function that drives producer revenue stream, product quality is also important for driving economic and social return. Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology. The biofortification process for improving the food quality of cassava has been very successful, particularly in ß-carotene content. However, adverse environmental effects such as low nitrogen (N) availability may impose severe constraints on the exploitation of this important alternative crop for family farms (Ngaboyisonga et al. 2012).

Unfortunately, in the humid tropics, high temperatures and rainfall combined with soils derived from clastic sedimentary rocks result in unfavorable conditions for organic matter accumulation and thus N availability. In particular, a continuous crop of cassava in the same area reduces nutrient availability and results in the depletion of soil fertility. These factors are now increasingly recognized as the fundamental causes of deforestation and decline food security in small landholder farms in the Amazon region and its surroundings (Aguiar et al. 2011). To circumvent these obstacles, farmers in the pre-Amazon region practice itinerant agriculture that is associated with the slashing and burning of natural vegetation where cassava plays an important role due to its tolerance for low-input systems. Unfortunately, this system has negative effects on the local and global environment and no longer provides social benefits to rural communities (Moura et al. 2014).

No-tillage alley cropping can be an efficient strategy for maintaining productivity in the low-fertility soils of humid tropical regions because this practice increases the availability and efficient use of nutrients. This system can be described as the planting of trees or shrubs in 2 or more sets of single or multiple rows. Before and during the cropping period, the leguminous branches are periodically pruned and laid down on the soil surface between the leguminous tree sets (Aguiar et al. 2010).

The efficiency of this system can be increased with the combined use of low- and high-quality residues of different leguminous tree species. First, soil rootability is enhanced by low-quality durable residues that increase nutrient uptake and nutrient use efficiency; second, the application of high-quality residues increases nutrient recycling, which enhances nutrient availability. However, trees also modify the biophysical environment in favor of their own growth preferences. Therefore, various negative or antagonistic interactions, both competitive and allelopathic, may influence the crop components of agroforestry systems (Moura et al. 2013).

Therefore, we hypothesized that the quality and quantity of cassava roots can be modified by green manure with the combined use of low- and high-quality leguminous residues in a humid tropic environment. Thus, the overall aim of this study was to test this hypothesis and identify a combination of leguminous residues of low/high quality that increase yield and the contents of essential human nutrients in cassava roots.

MATERIAL AND METHODS

Experimental site and trial establishment

The study area is located in the municipality of São Luís, in the northeast of the Maranhão State, at the following geographic coordinates: lat 2°30′S and →

long 44°18′W. The region has a hot, semi-humid, equatorial climate with a mean precipitation of 2,100 mm·year^{-1} and 2 well-defined seasons: a rainy one that extends from January to June and a dry season with a pronounced water deficit from July to December. The average temperature is approximately 27 °C, the maximum one is 37 °C, and the minimum temperature is 23 °C. The local soil is classified as an Arenic Hapludult and consists of 260 g·kg^{-1} of coarse sand, 560 g·kg^{-1} of fine sand, 80 g·kg^{-1} of silt, and 100 g·kg^{-1} of clay. The soil is derived from sedimentary clastic rock with a low carbon content and a high fine sand content; therefore, it has hardsetting characteristics (Moura et al. 2009) and low natural fertility (Table 1).

We tested 4 perennial leguminous species, 2 of which have a higher quality residue, i.e., *Leucaena leucocephala* (*Leucaena*) and *Gliricidia sepium* (*Gliricidia*), and 2 that produce a lower quality residue, i.e., *Clitoria fairchildiana* (*Clitoria*) and *Acacia mangium* (*Acacia*) (Vanlauwe et al. 1997; Aguiar et al. 2010). The quality of the legume residue was determined based on its relative nutrient (especially N), lignin, and polyphenol contents (Tian et al. 1995). The experiment was designed as a 6 × 2 factorial (a combination of 4 legume species *versus* 2 N levels) with 4 replications in a randomized block design and the following treatments: *Gliricidia* + *Acacia* (GA), *Gliricidia* + *Leucaena* (GL), *Gliricidia* + *Clitoria* (GC), *Leucaena* + *Acacia* (LA), *Leucaena* + *Clitoria* (LC), and a control without legumes. The chemical characteristics of the legumes are presented in Table 2.

The no-tillage alley cropping system was established in 2002 with legumes planted at a 0.05-m spacing in an eastern-western row orientation to maximize light absorption by the crops. Double rows were used such that each 5 × 4 m plot received 2 types of residues, i.e., low- and high-quality residues that resulted from the combination of 2 legumes (Figure 1). Since 2003, maize (*Zea mays* L.) was grown during the rainy season.

Table 1. Chemical analysis of soil in the experimental area, in depths of 0 – 10 and 10 – 20 cm.

Depth (cm) 0 - 10	Bare soil	*Leucaena* + *Clitoria*	*Gliricidia* + *Leucaena*	*Leucaena* + *Acacia*	*Gliricidia* + *Acacia*	*Gliricidia* + *Clitoria*
pH ($CaCl_2$)	5.20 ± 0.22[1]	5.03 ± 0.04	5.23 ± 0.15	4.81 ± 0.34	4.66 ± 0.18	5.45 ± 0.39
Al^{3+} ($mmol_c \cdot dm^{-3}$)	-	-	-	-	-	-
Ca^{2+} ($mmol_c \cdot dm^{-3}$)	9.00 ± 0.00	13.50 ± 0.58	11.25 ± 0.96	11.50 ± 0.58	10.75 ± 1.26	11.50 ± 1.00
Potential acidity ($mmol_c \cdot dm^{-3}$)	15.50 ± 1.00	25.58 ± 0.69	22.75 ± 2.22	28.50 ± 3.11	30.75 ± 9.22	15.75 ± 1.26
Sum of bases ($mmol_c \cdot dm^{-3}$)	17.24 ± 0.04	22.23 ± 0.05	19.27 ± 0.01	20.23 ± 0.01	15.23 ± 0.02	20.21 ± 0.05
Base saturation percentage (%)	50.09 ± 0.44	45.83 ± 0.36	45.46 ± 0.40	40.84 ± 0.36	31.43 ± 0.15	53.21 ± 0.83
Depth (cm) 10 - 20	**Bare soil**	***Leucaena* + *Clitoria***	***Gliricidia* + *Leucaena***	***Leucaena* + *Acacia***	***Gliricidia* + *Acacia***	***Gliricidia* + *Clitoria***
pH ($CaCl_2$)	4.18 ± 0.11	4.20 ± 0.04	4.19 ± 0.16	4.10 ± 0.10	3.99 ± 0.13	4.26 ± 0.09
Al^{3+} ($mmol_c \cdot dm^{-3}$)	3.13 ± 0.25	3.57 ± 0.08	3.45 ± 0.08	3.75 ± 0.11	6.40 ± 0.27	2.57 ± 0.08
Ca^{2+} ($mmol_c \cdot dm^{-3}$)	3.00 ± 0.00	3.02 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.97 ± 0.05	3.00 ± 0.01
Potential acidity ($mmol_c \cdot dm^{-3}$)	28.75 ± 1.50	36.00 ± 0.00	33.25 ± 0.50	33.25 ± 0.96	37.00 ± 2.16	29.50 ± 1.00
Sum of bases ($mmol_c \cdot dm^{-3}$)	6.10 ± 0.04	6.15 ± 0.04	6.02 ± 0.03	4.12 ± 0.03	7.15 ± 0.03	6.15 ± 0.05
Base saturation percentage (%)	14.41 ± 0.70	14.27 ± 0.27	13.29 ± 0.24	13.07 ± 1.71	13.22 ± 1.57	14.40 ± 0.42

[1]Mean plus standard error.

Table 2. Chemical characteristics of the leguminous.

	C/N	N	P	K	Ca	Mg
	kg·ha^{-1} of dry matter					
Leucaena	11.48	43.56	2.71	5.72	3.20	3.69
Gliricidia	13.51	37.01	1.48	4.62	3.36	2.33
Clitoria	18.38	27.21	3.15	4.89	3.75	2.09
Acacia	23.45	21.32	2.57	5.22	2.99	2.99

The first pruning of legumes was performed in 2004 and was continued in the following years. Pruning was performed annually to 0.5 m above ground level, and a second pruning was performed when needed to avoid shading the annual crop in the alleys. The green biomass produced annually by the legumes was similar (± 10 t·ha^{-1}) and evenly distributed over the respective plots. The weeds were controlled by using hand hoeing. The residues did not produce any differences in the density and richness of the weed species, as reported by Moura et al. (2014).

Pro-vitamin-biofortified cassava (cultivar BRS Dourada) was planted during the rainy season in February 2012 between rows of leguminous plants in plots measuring 5.0 m × 4.0 m. Cassava rows were spaced 1 m apart with 0.50 m between individual plants.

Cassava was fertilized at planting with 40 kg·ha^{-1} of K_2O and 70 kg·ha^{-1} of P_2O_5 applied as potassium chloride and triple superphosphate, respectively. In addition, 100 kg·ha^{-1} of urea N was applied twice in March 2012 and 2013 to the N treatments. The cassava was harvested in October 2013 after 20 months growth.

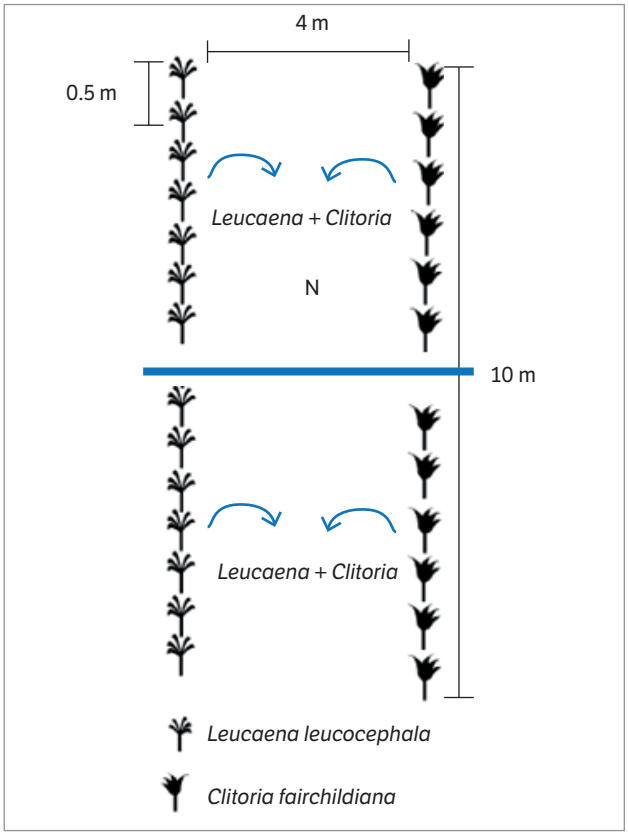

Figure 1. Diagram of an experimental plot showing a single row of trees, with and without nitrogen (N).

Soil sampling and analysis

Prior to planting the cassava, soil samples were collected from the 0 – 20 cm layer using a heavy duty auger. The soil samples were analyzed for pH (0.01 M $CaCl_2$ suspension, 1:2.5 soil/solution, v/v), organic carbon (Walkley-Black), P and exchangeable K, Ca, Mg (resin), and H + Al (SMP method) in accordance with the standard methods (van Raij et al. 2001). The cation exchange capacity (CEC) was determined as the sum of K, Ca, Mg, H, and Al. The sum of bases (SB) was calculated as K + Ca + Mg, and the base saturation percentage (BSP), as SB/CEC × 100.

The following yield parameters were measured on 15 cassava plants per plot: shoot weight (t·ha^{-1}), number of roots per plant, root weight (g), and total root production (t·ha^{-1}).

Three cassava roots were used for the following analyses:

- Proximate composition: cassava roots were peeled and crushed for determination of the moisture, and ash contents and were dried at 50 °C for 24 h for the determination of the lipid and protein contents. The moisture, ash and protein contents (AOAC 2005), and lipids (Bligh and Dyer 1959) were measured. The total carbohydrate content was estimated by subtracting 100 from the percent moisture, ash, lipid, and protein contents. Proximate composition is a partitioning of compounds in a feed into categories based on the chemical properties of the compounds, which are expressed as the content (%) in the feed, respectively.
- Starch content: cassava roots were dried in an oven at 50 °C for 24 h, and enzymatic hydrolysis was conducted (AOAC 2005). Root samples (100 mg) were added to 0.2 mL of 80% ethanol, 3 mL of 0.2 M sodium phosphate buffer (pH 6.8), and 100 µL of thermostable α-amylase. The mixture was placed in a water bath at 95 °C for 5 min. Then, 4 mL of 200 mM sodium acetate buffer (pH 4.5) and 100 µL of amyloglucosidase were added. The mixture remained at 50 °C in a water bath with stirring for 30 min. The contents were transferred to a 100-mL flask, filled to volume with water, and filtered through filter paper. The glucose content was determined using a Glucox 500 kit and converted to starch by multiplying it by a factor of 0.9.
- Mineral content: cassava roots were peeled, crushed, and dried at 50 °C for 24 h. Samples (1 g) were

digested at 200 °C for 1 h in 10 mL of nitric acid solution: perchloric acid (2:1) (AOAC 2005). After transferring the samples to a 50 mL volumetric flask that was then filled to volume with water, the elements were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP Optical Emission Spectrometer, Varian, Melbourne, Australia) and ICP Expert II software (Varian, Melbourne, Australia). The equipment operating conditions were as follows: plasma power of 1.0 kW; auxiliary gas (air) flow of 1.5 $L \cdot min^{-1}$; nebulizer pressure of 200 kPa, and plasma flow of 15.0 $L \cdot min^{-1}$.

- Carotenoid and pro-vitamin A contents: a carotenoid analysis was conducted with the protection from light, the avoidance of high temperatures, and the use of antioxidants (Rodriguez-Amaya and Kimura 2004). Briefly, the carotenoids from 20 g of cassava, to which butylated hydroxytoluene (0.1%) was added to minimize oxidation, were extracted 4 times with cold acetone using a Polytron homogenizer until the residue lacked color completely. The pigments were transferred from the acetone to petroleum ether. An aliquot of carotenoid extract was properly diluted, and the total carotenoids were determined spectrophotometrically at an absorbance of 450 nm using a UV-visible recording pectrophotometer (Lambda 25 Perkin Elmer) as well as the absorption coefficient of β-carotene (2592).

The extract was evaporated under N, dissolved in 1 mL of acetone, filtered through a 0.45 µm polytetrafluoroethylene syringe filter (Millipore), and then 10 µL was automatically injected into the high-performance liquid chromatography (HPLC) system. The HPLC-PAD (high-performance liquid chromatography-photodiode array detector) analysis was carried out using a ProStar Varian separation module equipped with a quaternary pump and autosampler injector controlled by a Galaxie workstation. A monomeric C_{18} column (Waters Spherisorb ODS-2; 3 µm; 150 × 4.6 mm) was used. The mobile phase consisted of acetonitrile (containing 0.05% of triethylamine), methanol, and ethyl acetate in proportions of 95:5:0 to 93:7:0 for 7 min, then 60:20:20 between 20 and 38 min, and finally 95:5:0 until the end of the run (42 min). The flow rate was 0.5 $mL \cdot min^{-1}$.

Carotenoid detection occurred at the wavelengths that corresponded to the maximum absorption of the carotenoids in the mobile phase. The carotenoids were identified according to: (a) their retention time, using carotenoid standards analyzed under the same chromatographic conditions; (b) their maximum absorbed wavelength (λmax) on a diode array detector; (c) their fine structure — % III/II, which is expressed as the ratio between the peak height of the longest-wavelength absorption band (III) and the middle absorption peak, generally λmax (II), which assumes the minimum between the 2 peaks as the baseline, multiplied by 100; and (d) the presence (or absence) of the *cis* peak between 340 and 346 nm.

The concentration of each carotenoid identified was measured using external standardization. Standard curves were prepared using 6 different concentration points for lutein (r = 0.999), β-carotene (r = 0.999), zeaxanthin (r = 0.999), and ζ-carotene (r = 0.999). The standards were obtained from the DSM Nutritional Product. *Cis* β-cryptoxanthin was estimated using β-carotene standard curves. The pro-vitamin A was calculated according to the new conversion factor specified by the Institute of Medicine Food and Nutrition Board (IOM 2001), where 12 µg of β-carotene and 24 µg of *cis* β-carotene and β-cryptoxanthin correspond to 1 retinol activity equivalent (RAE).

Statistical analysis

The data were subjected to analysis of variance with 2 factors followed by Duncan's multiple range test at 5%. Statistical analysis was performed using Statistica, version 10.0.

RESULTS AND DISCUSSION

The residues had a greater effect than the N, which did not influence any yield component when used alone (Table 3). There was large variation in the growth of cassava, and the residues had a marked effect on the dry matter production of the shoot. Except for LC and GA, all other combinations of residues were superior to the N treatment without residue. There was no significant difference between the N treatments in bare soil and the control. The LC + N treatment resulted in a shoot dry weight that was superior to the other treatments except for GC + N and GL + N. In the GL, LA, and GA treatments, there was no significant increase in dry matter with the use of N. The GA + N treatment resulted in a lower number of roots compared with the GC + N as well as LC + N treatments and was not significantly different from →

the control. The number of roots in the GC + N treatment was twice that of the control. The differences in the average root weight were small; the bare soil treatment with N had a lower root weight than GL + N, LC + N, GA + N, GL, and LA; other treatments were intermediate in their effect on root weight. The GL treatment was superior to the GC + N, GA + N, LC, GC, and GA treatments in increasing root production, even without N. The GC and LC treatments without N did not increase root production compared with the control. The addition of N increased the root production only in GC and LC treatments.

The low inorganic N agronomic efficiency (Δ productivity/N applied) in bare soil, which was verified in this experiment, has been reported as a significant problem in tropical agriculture (Moura et al. 2009). However, the surface application of residues improves the condition of soil rootability by increasing the depth layer for root growth, which is associated with greater water retention, root penetration, and nutrient uptake. It is worth highlighting that the combination of *Gliricidia* plus *Leucaena*, both high-quality residues, was superior for root yield. This indicates that the greater availability of organic N provided by faster decomposition of these legumes at the beginning of the rainy season was more important than the durability of the soil cover for root production. By contrast, the treatments with *Clitoria* (GC and LC) were more dependent on the use of N due to the low capacity of this species to provide the nutrient (Moura et al. 2010).

The variations in the proximate composition were small (Table 4). The roots of the control plots had a higher moisture content than the GC + N and BS + N treatments. The other treatments did not show significant differences. The lipid content in the GC + N treatment was higher than in the other treatments except for LC, BS + N, and the control. In addition, the protein content was higher in GC + N than in LC + N, LA + N, and the other treatments without N. Further, higher starch contents were measured in the GC + N and BS + N treatments, but the GL + N one was superior to all other treatments except for LC with and without N. There were no significant treatment effects on the ash and carbohydrate contents.

The residues affected the mineral nutrient content of cassava roots (Table 5). The highest calcium (Ca) and →

Table 3. Yields components for the different treatments of biofortified cassava.

	GL + N	LA + N	LC + N	GC + N	GA + N	GL	LA	LC	GC	GA	BS + N	Control
Weight of shoots ($t \cdot ha^{-1}$)	22.3abc	19.7bcd	26.6a	23.3ab	16.1def	20.1bcd	17.6cde	10.9fg	16.2def	13.1efg	8.3gh	5.4h
Number of roots per plant	5.2abc	5.2abc	5.3ab	5.5a	3.8cde	4.8abc	4.7abc	4.0bcd	3.8cde	3.3de	3.1de	2.6e
Roots weight (g)	334.4a	268.6ab	335.7a	313.0ab	342.7a	334.1a	326.5a	254.8ab	282.2ab	293.9ab	199.4b	317.7ab
Root production ($t \cdot ha^{-1}$)	33.7a	27.3abc	25.5bc	23.5c	22.6c	33.1ab	26.2abc	10.9e	14.0de	19.3cd	10.4e	9.6e

Distinct letters, in the same row, indicate significant differences by Duncan's test ($p < 0.05$). GL = *Gliricidia* + *Leucaena*; N = Nitrogen; LA = *Leucaena* + *Acacia*; LC = *Leucaena* + *Clitoria*; GC = *Gliricidia* + *Clitoria*; GA = *Gliricidia* + *Acacia*; BS = Bare soil.

Table 4. Proximate composition for the different treatments of biofortified cassava.

Components (%)[1]	GL + N[b]	LA + N	LC + N	GC + N	GA + N	GL	LA	LC	GC	GA	BS + N	Control
Moisture	58.6ab	62.2ab	60.3ab	57.3b	60.8ab	63.0ab	60.2ab	59.1ab	61.4ab	63.0ab	57.3b	64.0a
Ash	0.54	0.63a	0.66a	0.58a	0.57a	0.57a	0.56a	0.66a	0.63a	0.62a	0.64a	0.59a
Lipids	0.04f	0.07ef	0.07ef	0.16a	0.10bcde	0.09cde	0.10bcde	0.14ab	0.09cde	0.08def	0.12abcd	0.13abc
Protein	0.29ab	0.22cd	0.23bcd	0.32a	0.26abc	0.21cd	0.20cd	0.16d	0.22cd	0.19cd	0.26abc	0.17d
Starch	35.7b	33.9c	34.7bc	38.8a	34.0c	31.5d	33.5c	34.6bc	33.5c	30.1e	38.0a	30.6de
Carbohydrates	40.5a	36.9a	38.7a	41.6a	38.3a	36.1a	38.9a	39.9a	37.7a	36.1a	41.7a	35.1a

[1]Quantifications based on fresh weight basis. Distinct letters, in the same row, indicate significant differences by Duncan's test ($p < 0.05$). GL = *Gliricidia* + *Leucaena*; N = Nitrogen; LA = *Leucaena* + *Acacia*; LC = *Leucaena* + *Clitoria*; GC = *Gliricidia* + *Clitoria*; GA = *Gliricidia* + *Acacia*; BS = Bare soil.

magnesium (Mg) contents were measured in control plots; however, the GA + N treatment exhibited higher Ca contents compared with the other treatments. The potassium content varied with treatment and was higher in GC + N than in LA + N, LC + N, LA, GL, BS + N, and the control. The iron content was higher in GL + N than in the treatments without residues, BS + N, LA + N, and LC + N. The iodine content decreased in the following order: GL + N = GC + N > LA + N = LC + N = LA = GA. There was no treatment effect on the P or Zn contents.

The Ca and K contents varied with residue treatment. Vigorous growth of root systems is required to intercept and absorb these nutrients, and, therefore, Ca and K absorption is highly dependent on root activity (Sawyer and Mallarino 2002). However, other factors in addition to soil rootability and nutrient availability must have contributed to the higher Ca content in GA + N and the higher K content in GC + N. The combined use of 2 allelopathic genera, such as *Leucaena* and *Acacia* in LA treatment, may decrease the nutrient uptake of sensitive crops and eliminate the positive effects of the trees on nutrient availability and soil rootability (Moura et al. 2014). Therefore, the inclusion of a non-allelopathic (e.g., a native such as *Clitoria*) genus with additional desirable characteristics for residue mixtures could be a good potential strategy. This approach would help to mitigate the negative effect of allelopathic genera such as *Leucaena* and *Acacia*, where phenolic acids are present at very high concentrations and can alter the rate at which ions are absorbed by plants (Zhang et al. 2010). One mechanism through which plant growth is inhibited by this class of allelochemicals may be an alteration in membrane permeability (Glass and Dunlop 1974).

The low Fe and Zn contents in all treatments suggest the importance of a strategy for enhancing the uptake of these nutrients by crops in the infertile soils of the humid tropics. These nutrients can be chelated by soluble organic matter compounds during the decomposition process of residues. For example, organic matter containing Zn must undergo mineralization before it becomes available for plant uptake (Tarighi et al. 2012). The higher contents of iron in the GL + N treatment may be related to the faster decomposition process of this high-quality residue.

Due to their low concentration levels, the differences in lipid and protein contents in this experiment are not biologically relevant and therefore could not significantly modify root quality. In addition, the variation in lipid content, i.e., lower contents in the more productive treatments, appears to be related to the differences in productivity rather than the effect of the treatments, likely due to a dilution effect.

The inorganic N was more important than the residue for increasing the protein content in the roots; therefore, the accumulated protein (97.7 kg·ha^{-1}) in GL + N was superior to the treatments without inorganic N (data not showed). When a plant is presented with a large amount of N, the protein production increases and carbohydrate production is reduced; however, when N levels limit photosynthesis, proteins are not fully used in the synthesis of organic N compounds, and sugars accumulate (Worthington 2001). The higher content of starch in the GC + N treatment can be attributed to the greater capacity of cassava to take up potassium (K). Because cassava is a high carbohydrate producer, it requires a large amount of K, which has a special role in carbohydrate synthesis and translocation. Abundant uptake of K favors photosynthesis. The translocation of photosynthates from the green parts of the plant (leaf) to the storage root is of utmost importance for the building up of storage organs (tubers) (Susan John et al. 2010).

Table 5. Concentrations of minerals for the different treatments of biofortified cassava.

Minerals (mg·kg^{-1})[1]	GL + N	LA + N	LC + N	GC + N	GA + N	GL	LA	LC	GC	GA	BS + N	Control
Ca	46.0c	39.0c	35.0c	52.0c	116.0b	45.0c	50.0c	35.0c	46.0c	51.0c	36.0c	187.0a
Mg	65.0b	43.0b	47.0b	54.0b	68.0b	53.0b	43.0b	55.0b	53.0b	45.0b	63.0b	93.0a
K	132.0a	75.0c	83.0c	141.0a	112.0b	94.0bc	68.0c	106.0b	124.0ab	94.0c	77.0c	104.0b
P	170.0a	114.0a	125.0a	162.0a	139.0a	143.0a	120.0a	132.0a	152.0a	122.0a	135.0a	163.0a
Zn	0.9a	0.8a	0.4a	0.7a	1.0a	0.8a	0.4a	0.8a	0.7a	0.6a	0.5a	0.9a
Fe	3.5a	2.1bc	1.5c	2.7abc	2.8ab	2.0bc	1.7bc	2.0bc	2.1bc	2.2bc	2.2bc	1.9bc
I	16.0a	11.0b	11.0b	16.0a	13.0ab	12.0ab	11.0b	12.0ab	13.0ab	11.0b	12.0ab	14.0ab

[1]Quantifications based on fresh weight basis. Distinct letters, in the same row, indicate significant differences by Duncan's test ($p < 0.05$). GL = *Gliricidia* + *Leucaena*; N = Nitrogen; LA = *Leucaena* + *Acacia*; LC = *Leucaena* + *Clitoria*; GC = *Gliricidia* + *Clitoria*; GA = *Gliricidia* + *Acacia*; BS = Bare soil.

In the biofortified cassava genotype (BRS Dourada), *trans* β-carotene was the predominant isomer in all treatments and the major isomer with vitamin A activity (Figure 2). The carotenoid content was modified by the application of residues (Table 6). The highest total carotenoid content was measured in the roots of the GC + N and LC treatments. GC + N was superior in terms of *cis* β-cryptoxanthin, *trans* β-carotene, and (13 *cis*) β-carotene, whereas LC was superior in terms of *cis* β-cryptoxanthin, *trans* ζ-carotene, (9 *cis*) β-carotene, and (13 *cis*) β-carotene. The highest pro-vitamin A content was measured in GC + N, followed by LC and the remaining treatments.

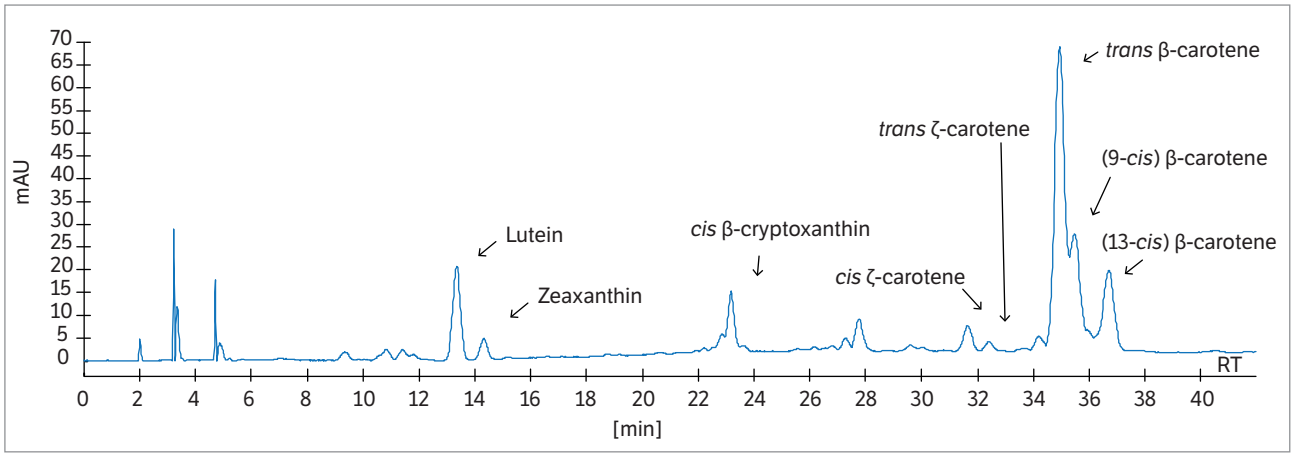

Figure 2. Chromatogram of the carotenoids of the control.

Table 6. Contents of carotenoids and pro-vitamin A for the different treatments of biofortified cassava.

Carotenoids (mg·kg^{-1})[1]	GL + N	LA + N	LC + N	GC + N	GA + N	GL
Lutein	0.04d	0.04d	0.04d	0.05cd	0.04d	0.12a
Zeaxanthin	0.01b	0.01b	0.01b	0.02b	0.01b	0.02b
cis β-cryptoxanthin	0.08b	0.06cd	0.04ef	0.11a	0.05de	0.07bc
cis ζ-carotene	0.05c	0.05c	0.06b	0.06b	0.04d	0.05c
trans ζ-carotene	0.03b	0.03b	0.04a	0.03b	0.04a	0.04a
trans β-carotene	0.44cd	0.43cde	0.32e	1.08a	0.44cd	0.41cde
(9-*cis*) β-carotene	0.25cd	0.17fgh	0.12hi	0.37b	0.11i	0.19ef
(13-*cis*) β-carotene	0.18c	0.14cd	0.11d	0.35a	0.13cd	0.14cd
Total	1.6c	1.2e	1.2e	2.1a	1.4d	1.3d
Pro-vitamin A (RAE per 100g)	5.7d	5.2de	3.8f	12.4a	4.9def	5.1def
Carotenoids (mg·kg^{-1})[1]	**LA**	**LC**	**GC**	**GA**	**BS + N**	**Control**
Lutein	0.04d	0.08bc	0.04d	0.12a	0.10ab	0.14a
Zeaxanthin	0.01b	0.02b	0.01b	0.02b	0.02b	0.04a
cis β-cryptoxanthin	0.03f	0.10a	0.07bc	0.06cd	0.05de	0.05de
cis ζ-carotene	0.06b	0.06b	0.05c	0.05c	0.07a	0.06b
trans ζ-carotene	0.04a	0.04a	0.03b	0.03b	0.04a	0.04a
trans β-carotene	0.48c	0.76b	0.77b	0.35de	0.43cde	0.46cd
(9-*cis*) β-carotene	0.28c	0.46a	0.22de	0.14ghi	0.17fgh	0.16fgh
(13-*cis*) β-carotene	0.17c	0.32a	0.26b	0.11d	0.15cd	0.11d
Total	1.3d	2.0b	1.7c	1.1e	1.3d	1.7c
Pro-vitamin A (RAE per 100g)	6.0d	10.0b	8.7c	4.2ef	5.1def	5.2de

[1]Quantifications based on fresh weight basis. Distinct letters, in the same row, indicate significant differences by Duncan's test ($p < 0.05$). GL = *Gliricidia* + *Leucaena*; N = Nitrogen; LA = *Leucaena* + *Acacia*; LC = *Leucaena* + *Clitoria*; GC = *Gliricidia* + *Clitoria*; GA = *Gliricidia* + *Acacia*; BS = Bare soil; RAE = Retinol activity equivalent.

The highest carotene and pro-vitamin A contents were measured in the GC + N treatment that also contained a high protein content, which may be attributed to the protein required for the accumulation of carotenoids in cassava. Genetic studies on total carotenoid and protein contents indicate a highly significant and positive correlation between root protein in the plastid fraction and total carotenoid content (Carvalho et al. 2011). The current experiment indicated that changes in protein content caused by environmental factors such as soil fertility maintain this relationship.

CONCLUSION

In soils of low natural fertility in the humid tropics, the quality and yield of cassava can be modified with residues of leguminous trees; however, N fertilizer does not produce the same effect. In addition, to increase root production, the use of combined high-quality residues with the fastest release of N (GL) was superior to residues with slow decomposition rates (GA and GC). The increase in protein content was dependent on readily available N and influenced the carotenoid content. The allelopathic effect of exotic genera such as *Leucaena* and *Acacia*, especially when combined, can decrease the uptake of nutrients such as potassium and therefore reduce starch accumulation. In soil derived from sedimentary clastic rock with low contents of iron and zinc, a strategy to increase the contents of these nutrients in cassava remains to be discovered.

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