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MINERAL NUTRITION, GROWTH AND YIELDS OF ANNATTO TREES (*BIXA ORELLANA*) IN AGROFORESTRY ON AN AMAZONIAN FERRALSOL

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SUMMARY

Permanent tree crop agriculture and multi-strata agroforestry are among the most promising options for the agricultural use of the mostly nutrient-poor and physically fragile soils of central Amazonia. In general, though, information on the optimum management of local tree crops under these conditions is inadequate. Annatto (*Bixa orellana*) is a small tree of tropical American origin whose seeds contain a non-toxic, carotenoid dye that is used widely in food and cosmetic products. The authors studied seed yields at ages four to seven years, biomass at seven years, nutrient accumulation and mineral nutrition of annatto trees growing in multi-strata agroforestry with different inputs of fertilizer and lime on a xanthic Ferralsol in central Amazonia. Leaf samples of three age classes were collected four times during one year, and nutrient concentrations were related to soil nutrient status, growth and yield of the trees in order to develop an optimum sampling scheme for foliar analysis. Growth and yields of the trees showed a pronounced response to increased fertilizer and lime input, which seemed to be due mainly to improved availability of phosphorus. Nitrogen fertilizer had no effect and may not be necessary for well-established trees on this soil. High litter quality and substantial nutrient recycling with annual pruning make annatto a valuable component for agroforestry systems. In this experiment, however, yields were low and decreased after the fifth year, presumably as an effect of infertile soil, shading by larger trees and, possibly, a negative effect of the drastic annual pruning with removal of the entire leaf and small-branch biomass on the vitality of the trees. Annatto is probably best suited for associations with small tree crops. Less drastic pruning treatments than those practiced in this experiment may be preferable.

INTRODUCTION

With the exception of a narrow strip of nutrient-rich alluvial soils along the rivers coming down the Andes, most of the Amazon basin is covered by highly weathered, acidic and often physically fragile soils with very limited potential for

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agriculture (Sombroek, 2000). In order to maintain as much as possible of the region under its primary forest cover, the development of sustainable, intensive crop production systems for already cleared areas is a necessary precondition. Permanent tree crop agriculture and multi-strata agroforestry based on tree crops are among the most promising options for this. Their further development, however, requires solid knowledge of the precise site requirements and management of tree crops suitable for Amazonian conditions. Unfortunately, this knowledge is often insufficient, even for the economically most promising of local tree crop species (Schroth *et al.*, 2001b).

Annatto (*Bixa orellana*) is an example of this situation. It is a small tree of tropical American origin whose red seeds contain carotenoids, especially bixine and norbixine, which are non-toxic and, therefore, widely used as dye for food and cosmetic products. Annatto dye has re-gained importance especially since the synthetic Red Dye No. 3 was banned by the US Food and Drug Administration in 1990 because of its carcinogenic properties (Smith *et al.*, 1992). Different parts of the plant are also used for a range of medicinal purposes and against agricultural and domestic pests (Bole, 1995; Revilla, 2000). Annatto has been cultivated in Latin America since pre-Colombian times and nowadays is produced in several tropical countries such as Brazil, Peru, Mexico, Indonesia, India and East Africa (Bole, 1995).

As with most indigenous tree crops of the Amazon region, however, very little is known about the nutritional requirements and the optimal agricultural management of annatto under Amazonian conditions (Falesi & Kato, 1992; Castro *et al.*, 1994). The situation is further complicated when the species is grown in association with other trees in agroforestry plantings. According to sources cited by Bole (1995) and Rosalen *et al.* (1991), annatto grows best on nutrient-rich clay soils with a pH of 5.5–6. Such soils, however, are hard to find in the region, suggesting that mineral and/or organic fertilization are important for the successful, long-term production of this crop. Smith *et al.* (1992), however, reported that in eastern Amazonia annatto was successfully grown on poor soil after many slash-and-burn cycles. Castro *et al.* (1994) stressed the importance of soil physical aspects: sufficient depth, permeability and absence of compact surface horizons. These conditions are satisfied by the typically deep and well-structured ferralitic soils of the region in their natural state, although soils that have been compacted through inappropriate management (*e.g.* intensive pasture use or mechanized clearing) may be less suitable for this crop.

The objective of this research was to provide basic information on seed yields, biomass, nutrient accumulation and mineral nutrition of annatto trees growing under agroforestry conditions with different inputs of fertilizer and lime on a central Amazonian Ferralsol. The most limiting nutrients for growth and yield of annatto on this soil type were to be identified, and an optimum sampling scheme for foliar analysis of this little-studied, but promising tree crop species was to be developed.

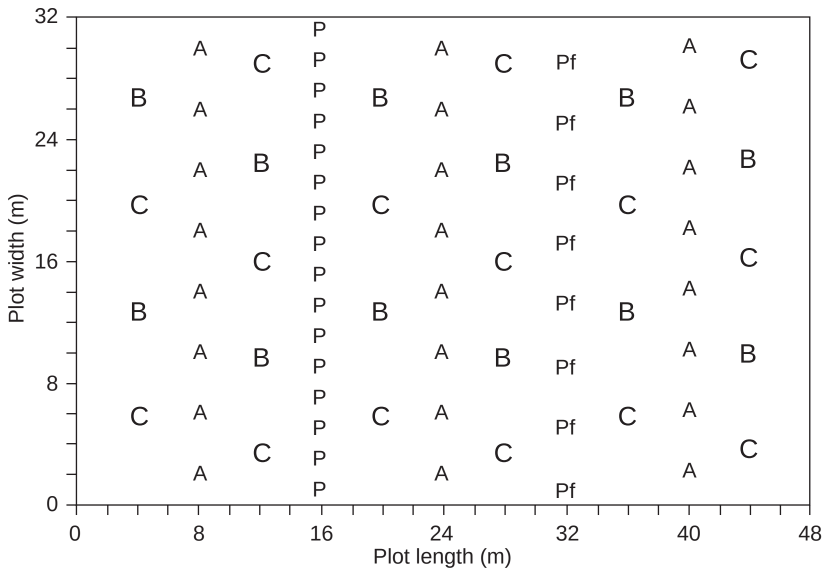

Figure 1. Layout of a multi-strata agroforestry system in central Amazonia. A, annatto; C, cupuaçu; B, Brazil nut trees; P, peach palm for palmito; Pf, peach palm for fruit.

MATERIALS AND METHODS

Study site and experiment layout

The study was carried out on the research station of Embrapa Amazônia Ocidental near Manaus in Brazilian Amazonia. The climate is lowland humid tropical with mean annual precipitation of 2600 mm. The driest months are July to September, and the wettest months are February to April. The soil is a xanthic Ferralsol with clayey texture and kaolinitic mineralogy. For detailed soil data see Schroth *et al.* (2000a).

A field experiment was established in February and March 1993 on an area of about 13 ha which first had been cleared for a rubber plantation in 1980, but this had been abandoned a few years later and the area developed into secondary forest. The experiment consisted of different agroforestry and monoculture systems with important tree crops of the region. In one of the agroforestry systems, annatto (*Bixa orellana*) was associated with peach palm (*Bactris gasipaes*), cupuaçu (*Theobroma grandiflorum*) and Brazil nut trees (*Bertholletia excelsa*, Figure 1). The annatto trees were planted in rows at 4-m spacing. During the first year, cassava (*Manihot esculenta*) was grown as a temporary intercrop between the trees, followed by a leguminous cover crop (*Pueraria phaseoloides*).

A few weeks after planting the annatto trees in the field, they were cut at 0.75-m height to induce branching. Branches that had formed at less than 0.4-m height were removed five months later. From the second year onwards, the fruits were harvested annually in the period from March to May. They were weighed in the field and sub-samples were taken from each plot and dried at 70 °C to constant

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Table 1. Fertilizer applied during seven years in multi-strata agroforestry plots with annatto trees (*Bixa orellana*) on a xanthic Ferralsol in central Amazonia.

	Fertilization level			
	low minus N	low	full	full plus P
	Fertilizer applied to annatto trees (g tree^{-1})			
Nitrogen[a]	50	138	424	424
Phosphorus[b]	80	80	266	343
Potassium[c]	231	231	769	769
	Fertilizer applied per plot (kg ha^{-1})[e]			
Nitrogen[a]	46	89	290	290
Phosphorus[b]	50	50	167	209
Potassium[c]	108	108	355	355
Dolomite[d]	56	681	2270	227

[a] As urea (45% N) until 1996, then as ammonium sulphate (21% N); in 'low minus N' no N was applied after May 1996.
[b] As triple super phosphate (22% P), except in 12/1996 (North Carolina Phosphate 'Atifos', 13% P).
[c] As potassium chloride (50% K).
[d] In 'low minus N' no dolomite was applied after May 1996.
[e] Includes fertilizer applied to associated tree crops and temporary intercrops.

weight before the seeds were extracted and weighed. Every year at the end of the harvest, the trees were cut back to 1.50 m height to increase fruit set in the following year, removing the small branches and leaf biomass. The prunings were left as mulch on the soil under the trees.

Mineral fertilizer was applied around the individual trees in December and June of each year. Initially, there were two fertilizer treatments whereby annatto and associated trees either were fertilized and limed according to local recommendations (full fertilization) or 30% of this amount was applied (low fertilization, Table 1). A second factor, whereby the plants in the nursery were either inoculated or not with mycorrhiza, had no effect on annatto yields in the first two years' harvests (1994 and 1995). This is in accordance with the findings reported by L. Gasparotto and H. Preisinger, (eds., unpublished project report, 1996) with similar degrees of root colonization with mycorrhiza of inoculated and non-inoculated plants in the field. The presence of two plots for each fertilizer level in each replication allowed the introduction of two additional fertilizer treatments in 1996: a full fertilization treatment with an additional 50% of phosphate fertilizer (full plus P), and a low fertilization treatment where no more nitrogen fertilizer and dolomitic lime were applied (low minus N). In 1993, the trees received 60 and 200 g of dolomitic lime in the planting hole in the low- and full fertilization treatments respectively. In 1996, 2.1 t ha^{-1} of dolomitic lime were broadcast in the full fertilization plots, the low fertilization plots received 30% of this quantity, and the low-minus-N plots were not limed. The manioc (cassava) intercrop received 20 kg ha^{-1} P as triple super phosphate and 38 kg ha^{-1} potassium (K) as potassium chloride in the full- and 30% of these values in the low fertilization plots. Total amounts of fertilizer and lime applied to the annatto trees and associated species during seven years are given in Table 1.

The plots were arranged in a randomized complete blocks design. Three replicate blocks with one plot per fertilizer treatment per block (12 plots in total) were included in the study. Plot size was 48×32 m, with 24 annatto trees per plot (Figure 1).

Estimation of above-ground biomass and nutrient contents

The estimation of the above-ground biomass of the trees was based on an inventory of the diameters of six trees at 0.20-m height in the centre of each plot, and the destructive harvesting of seven trees of different sizes from the whole experiment for the development of an allometric relationship. These activities were carried out between December 1999 and February 2000, when the trees had been in the field for seven years. The trees selected for destructive harvesting were cut at ground level and separated into stem, old branches, branches grown after the previous post-harvest pruning (subdivided into diameter classes >20, 10–20 and <10 mm) and leaves. Each fraction was weighed and representative samples collected for dry-matter determination by drying at 65 °C (leaves) or 105 °C (woody structures) to constant weight. To estimate the nutrient accumulation in the above-ground biomass of the trees, four trees of different sizes were selected and dried sub-samples of all tissue types were ground and digested according to Novozamsky *et al.* (1983). Nitrogen and P were measured colorimetrically, and K, Calcium (Ca) and Magnesium (Mg) by atom absorption spectrometry.

Effect of fertilizer levels on foliar and soil nutrient levels

Leaf samples were collected from all study plots in November 1997 (end of the dry season), January, May and August 1998 (beginning of the dry season), to evaluate the effect of the different fertilizer inputs on the mineral nutrition of the trees and to identify critical nutrient elements for this species and site. The leaves were collected from four sides of each tree at the middle of the crown height from six trees per plot. Leaves of three ages were sampled and analysed separately: the youngest green leaves, mature leaves from the middle part of a branch ('medium leaf'), and old leaves, still entirely green ('old leaf'), from the inner part of the branch (adaxial). From each position and age class, four leaves were collected. The leaves were washed in tap water, dried at 65 °C, ground, digested and analysed as described above.

Soil samples were collected from 0–200 mm depth under the annatto trees in April-May 1998, before the fertilization of the plots. After air-drying and sieving the soil to pass through a 2 mm grid, the following analyses were carried out: total N by Kjeldahl digestion; available P, K, Ca and Mg by extraction with the Mehlich 3 solution at a soil:solution ratio of 1:10 (Tran and Simard, 1993), followed by colorimetric measurement of P and measurement of K, Ca and Mg by atom absorption spectrometry; exchangeable acidity by extraction with 1 m KCl at a soil:solution ratio of 1:15 and titration with NaOH against a phenolphthaleine

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indicator; pH with a glass electrode at a soil:solution ratio of 1:2.5 in destilled water. The cation exchange capacity was calculated by summing the basic cations and the exchangeable acidity.

Statistical analysis

Statistical comparisons of yield, soil and foliar nutrient data were made by analysis of variance for a randomized complete plot design.

RESULTS

Growth and seed yields

The seven-year-old annatto trees showed a curvilinear relationship between basal area and above-ground dry matter with a saturation value around 23 kg per tree for basal areas above 130 cm^2 (Figure 2). Probably, this was a result of the complete annual pruning at 1.50 m height. Total above-ground dry matter (DM, in kg $tree^{-1}$) was related to basal area (BA, in cm^2) at 0.20 m height by:

$$DM = -21.7 + 0.505\ BA - 0.00143\ BA^2 \qquad (r^2 = 0.95).$$

On average, the above-ground biomass of the trees consisted to 68% (± 3) of the short trunk and the old branches which were left over after the annual crown pruning, 18% (± 2) of branches of <1 year which had re-grown since the previous year's pruning, and 14% (± 1) of leaves (Figure 2). The percentage of trunk and

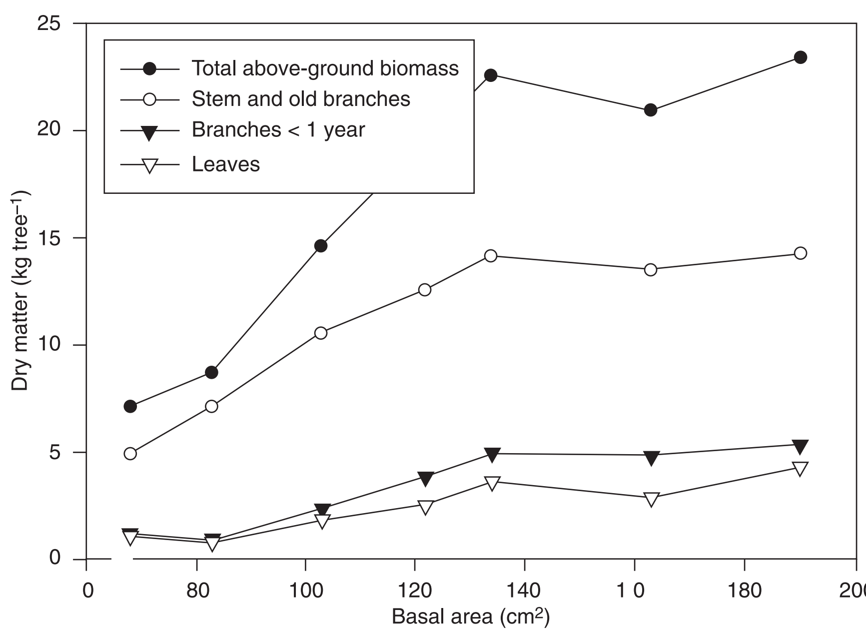

Figure 2. Contribution of different tissue types to the aboveground biomass of seven-year-old annatto trees (*Bixa orellana*) grown under agroforestry conditions on a xanthic Ferralsol in central Amazonia as influenced by tree size. Each data point on the x-axis represents one destructively harvested tree.

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Table 2. Yields and biomass of annatto (*Bixa orellana*) in a multi-strata agroforestry system at four fertilization levels on a xanthic Ferralsol in central Amazonia, and statistical probability of differences between treatments (*F*-test). The trees were planted in early 1993.

Year	Fertilization level				*s.e.d*	*P*
	low -N	low +N	full	full +P		
Above-ground biomass (without fruits, kg tree^{-1})						
2000	12.4	11.2	17.2	17.8	1.7	0.018
Yields (dry seeds, kg tree^{-1})						
1997	0.59	0.37	0.90	0.68	0.12	0.011
1998	0.67	0.59	1.00	0.94	0.13	0.029
1999	0.38	0.43	0.63	0.71	0.11	0.050
2000	0.29	0.33	0.54	0.51	0.11	0.111
Mean	0.48	0.43	0.77	0.71	0.09	0.015

old branches tended to increase and that of young branches tended to decrease with increasing tree size. The percentage of leaves was independent of tree size.

With the allometric equation given above, it was possible to estimate the above-ground dry weight of the other trees included in the inventory and to analyse the effects of the fertilizer treatments on tree growth (Table 2). This responded significantly to increased fertilizer application, with an average weight of 11.8 kg per tree in the two ‘low’ treatments and 17.5 kg per tree in the two ‘full’ treatments, without significant differences within these two groups. The growth response to fertilization was in accordance with the response of the seed yields, which were significantly greater in the ‘full’ than in the ‘low’ treatments in the years 1997–99 and on the average of the four study years (Table 2). For a more than three-fold increase in applied fertilizer, tree growth increased by 48% and yields increased by 65%. In general, however, annatto yields were rather low in this experiment compared with reported seed yields approaching 2 t ha^{-1} (Smith *et al.*, 1992), which corresponds to about 3 kg tree^{-1} if a normal planting density of 6–700 trees ha^{-1} is assumed. Yields also decreased by almost one-half from 1998, when the trees were five years old, to the year 2000 (Table 2).

Soil fertility

Despite fertilization and liming, the soil was acidic and had a base saturation of only about 20% in the ‘low’ and 40% in the ‘full’ fertilization treatments (Table 3). Base saturation was significantly less in the un-limed ‘low minus N’ than in the limed ‘low plus N’ treatment (Table 3); however, this difference did not (yet) affect tree growth and yields (Table 2). Available P was twice as high in the ‘full’ than in the ‘low’ treatments (Table 3). The additional P applied in the ‘full plus P’ plots did not affect the soil data at this sampling date although the effect was apparent in soil samples collected at other times of the year (M. S. S. Mota, unpublished). The P levels in the ‘low’ plots were low and those in the ‘full’ plots were medium according to reference values given by Havlin *et al.* (1999). Significant increases

Table 3. Nutrient availability in a xanthic Ferralsol in central Amazonia at 0–200 mm under annatto (*Bixa orellana*) at four fertilization levels in May 1998, and statistical probability of differences between treatments (*F*-test)

	Fertilization level					
	low -N	low +N	full	full +P	*s.e.d*	P
pH (H_2O)	4.35	4.27	4.38	4.23	0.05	0.095
P (mg kg^{-1})[a]	10.1	12.2	22.5	22.8	3.20	0.012
K (mmol+ kg^{-1})[a]	0.59	0.67	0.73	0.72	0.01	0.471
Ca (mmol+ kg^{-1})[a]	1.67	2.87	7.07	5.59	0.08	0.002
Mg (mmol+ kg^{-1})[a]	0.78	1.78	3.91	2.43	0.05	0.003
CEC (mmol+ kg^{-1})[a]	20.50	22.10	25.50	25.10	0.10	0.002
Base saturation (%)	15.00	24.00	46.00	35.00	4.00	<0.001

[a] extracted with Mehlich 3 extractant

Table 4. Nutrient concentrations (mg g^{-1}) in leaves of annatto (*Bixa orellana*) and associated tree and cover crops in a multi-strata agroforestry system on a xanthic Ferralsol in central Amazonia. Values are means of three leaf age classes.

	N	P	K	Ca	Mg
Annatto, August	41.4	3.6	15.6	7.7	3.5
Annatto, November to May[a]	27.3	2.0	13.0	7.8	3.4
s.e.d for sampling date	0.8	0.1	0.4	0.2	0.1
Cupuaću[b]	18.1	1.0	4.3	4.6	2.7
Brazil nut[b]	17.9	0.8	4.5	9.3	3.1
Peach palm[b]	29.7	1.8	9.7	3.1	2.2
Pueraria[b]	38.3	2.3	14.5	5.3	3.5

[a] means of three sampling dates
[b] means of four sampling dates

with increasing nutrient input were seen also in exchangeable Ca and Mg. Exchangeable K was not significantly affected by the treatments. Similarly, total N was 2.1 g kg^{-1} at 0–100 mm depth and 1.1 g kg^{-1} at 100–300 mm depth without significant differences between fertilizer levels (Schroth *et al.*, 2001b).

Foliar nutrient concentrations

The foliar nutrient concentrations of annatto showed a significant ($P<0.001$) seasonal pattern, with much higher concentrations of N and P and slightly higher concentrations of K in August, when the foliage was still young after the post-harvest pruning in April/May, than at the other sampling dates (Table 4). In general, the annatto foliage was relatively nutrient-rich compared with that of the associated tree crop species, especially cupuaçu and Brazil nut (Table 4). Peach palm had similar N and P contents as annatto during most of the year but, being a monocotyledonous species, had much lower foliar Ca and Mg concentrations. In August, the N and P contents of the annatto foliage were even higher than those of the leguminous cover crop, *Pueraria phaseoloides*.

In accordance with the soil data, the foliar N and K concentrations of annatto

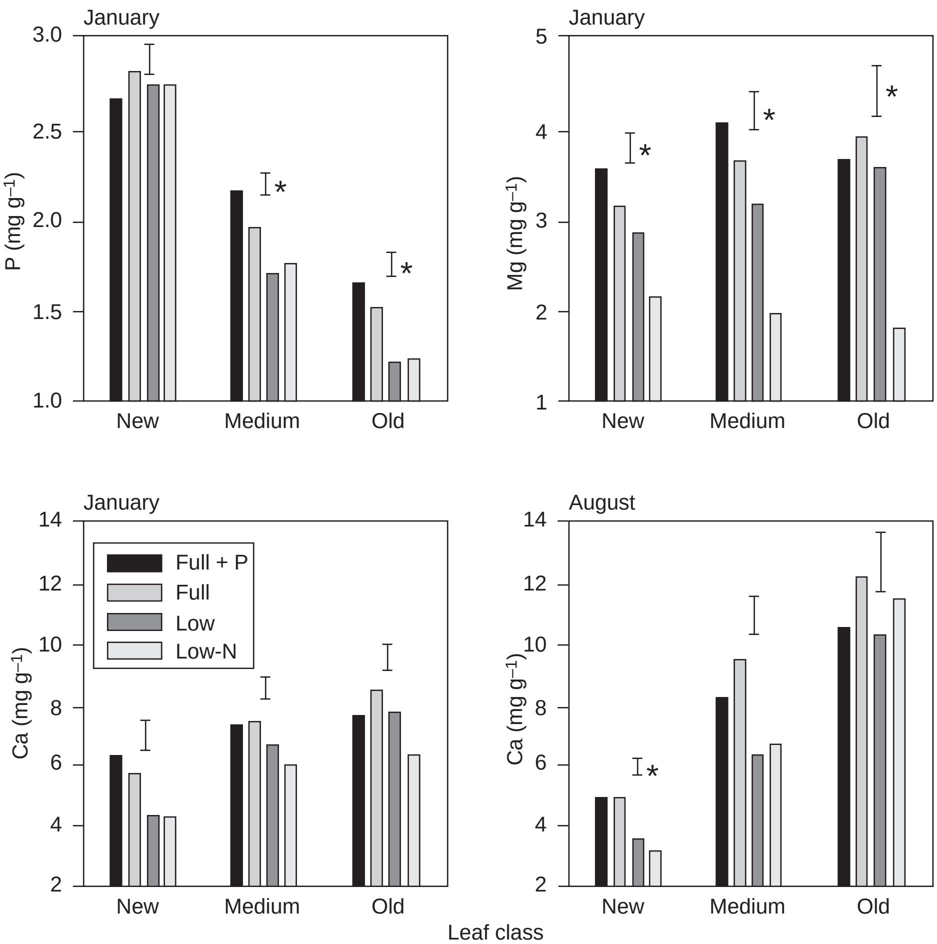

Figure 3. Phosphorus, magnesium and calcium concentrations in three leaf age classes of five-year-old annatto trees (*Bixa orellana*) grown under agroforestry conditions on a xanthic Ferralsol in central Amazonia as affected by fertilizer levels. Error bars correspond to one *s.e.d.* Asterisks indicate significant differences between fertilizer levels at P=0.05 (F-test).

did not respond significantly to the fertilizer treatments (data not shown). The different P fertilization levels and soil P contents were best reflected by the foliar P contents in January, at the beginning of the rainy season and shortly after the application of fertilizer (Figure 3). At this sampling date, the P contents were significantly higher in the 'full' than in the 'low' treatments both in the old and in the medium leaves. No fertilizer effect was observed in the young leaves, indicating that the plants compensated for reduced P supply through increased translocation from old to young leaves. In November and May the same tendencies could be seen as in January, but the fertilizer effect was only significant for the old leaves, whereas in August it was not significant for any leaf class (not shown).

The fertilization effect on foliar Mg concentrations was always significant for

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Table 5. Accumulation of dry matter and macronutrients in seven-year-old annatto plants (*Bixa orellana*) grown in a multi-strata agroforestry system on a xanthic Ferralsol in central Amazonia[a].

	Whole tree (with fruits)	Crown prunings	Whole fruits[b]	Seeds only[b]
Dry matter (kg tree^{-1})	18.9	5.6 (30%)	1.68 (9%)	0.54 (3%)
N (g tree^{-1})	175.0	78.9 (45%)	33.60 (19%)	10.60 (6%)
P (g tree^{-1})	17.7	6.4 (36%)	5.40 (31%)	2.70 (15%)
K (g tree^{-1})	94.1	33.8 (36%)	25.10 (27%)	10.04 (11%)
Ca (g tree^{-1})	57.5	28.2 (49%)	3.90 (7%)	0.90 (2%)
Mg (g tree^{-1})	24.4	8.7 (36%)	3.10 (13%)	1.50 (6%)

[a] Average of the two 'full' fertilization treatments. Values in brackets are percent of the whole tree (first column).

[b] Calculated from seed yields (Table 2), a seed:fruit ratio of 0.322 as measured in 2000, and fruit and seed nutrient concentrations as determined on samples collected at harvest time in 2001.

the medium leaves and also for either the young leaves (August) or the old leaves (November) or both (January and May, Figure 3). This effect was due mostly to the strongly reduced Mg concentrations in the un-limed 'low minus N' treatment compared with all other treatments. While, usually, the Mg concentration was somewhat lower in the young than in the old leaves, this trend was inverted in the 'low minus N' plots, indicating that limited Mg supply may have forced the trees to translocate Mg increasingly from the old into the young leaves. In agreement with the soil data, the foliar Ca concentrations were higher in the 'full' than in the 'low' treatments at all sampling dates except May, but the effect was only significant in the new leaves in August (Figure 3).

Nutrient contents of trees, prunings and harvest products

For an average tree in the 'full' fertilization treatments, 9% of the dry matter, containing 7–31% of the macronutrients, was removed with the harvest (Table 5). If the fruit shells are returned as mulch, as recommended by Falesi and Kato (1992), the nutrient export is reduced to 2–15% (see last column in Table 5). The biomass and nutrient removal with the crown prunings following the harvest was estimated from the weight and nutrient contents of all leaves and one-year-old branches. These corresponded to 30% of the dry matter of the trees, containing 45% of the total N, 49% of the Ca, and 36% of the P, K and Mg (Table 5). The prunings were left to decompose under the trees and there was thus no nutrient export associated with this management measure. Nevertheless, the harvest and subsequent crown pruning led to the removal of about 40% of the above-ground tree biomass, including the whole leaf biomass and >60% of the N, P and K reserves of the trees within two weeks (Table 5).

DISCUSSION

Under agroforestry conditions on a ferralitic Amazonian upland soil, annatto showed a pronounced growth and yield response to increased fertilizer inputs.

Foliar nutrient analyses suggested that improved P supply was the principal reason for increased growth and yields in the 'full' treatments, which is in agreement with the generally low P availability on this type of soil. According to the presented data, such responses to increased P fertilization would be expected when soil P (Mehlich 3) is around 10 mg kg^{-1} and when foliar P is around 1.7–1.8 mg g^{-1} in medium and 1.2 mg g^{-1} in old leaves. Differences in Ca supply may have had an additional influence on annatto yields, although Rosalen *et al.* (1991) reported little sensitivity of the species to a low supply of this element in nutrient solution tests. The fact that the two 'low' treatments differed strongly in foliar Mg concentrations but not in yield suggests that the Mg supply did not yet influence growth, although there were indications of a progressive development of latent Mg deficiency in the 'low minus N' treatment. Since the root system of annatto is considered sensitive to soil acidity (Falesi & Kato, 1992), it is likely that the higher lime level also improved growth and yields by reducing exchangeable Al in the soil (Table 3). Previous studies on this soil have shown that exchangeable Al is most likely to affect plant growth when soluble fertilizer is applied at high rates (Schroth *et al.*, 2000a).

Nitrogen and K had no influence on the growth and yield differences of annatto at this site, despite the suggestion by Castro *et al.* (1994) that these two elements most influence the flowering and fruit formation of the species. For N this was in accord with high N availability in the soil, resulting from high soil N mineralization rates and biological N fixation by the leguminous cover crop (Schroth *et al.*, 2001a). The absence of a yield response to N fertilizer has been shown also with several other tree crops at this site (Schroth *et al.*, 2000b; Schroth *et al.*, 2001b). The lack of a yield response to K could have been influenced by the generally low yield level.

The results suggest that an adequate supply of P fertilizer and dolomitic lime is particularly important for the production of annatto on this soil, whereas N fertilizer may not be necessary once the plants are well established. Based on qualitative observations in eastern Amazonia, Falesi and Kato (1992) suggested the reduction or dispensation of N fertilization of annatto from the second year. Potassium fertilizer should be applied at levels compensating for harvest exports (Table 5). In combination with soil analysis, foliar nutrient analysis seemed to be a useful tool for monitoring the effect of mineral fertilizer treatments on annatto. Guidelines for sample collection can be derived from the presented data (Table 6).

According to the high leaf-nutrient concentrations and the pronounced response to increased fertilizer input, annatto has to be considered a relatively nutrient-demanding species compared with other local tree crops such as cupuaçu with its much lower foliar nutrient concentrations and less pronounced response to fertilizer (Schroth *et al.*, 2001b). The high nutrient contents and rapid decomposition of annatto foliage also imply a beneficial effect of the species on nutrient cycles, especially as a considerable part of the above-ground biomass and nutrients contained in it are regularly recycled through the post-harvest pruning. In a study comparing the litter fauna under annatto, cupuaçu, Brazil nut, peach

Table 6: Combinations of leaf class and sampling date most sensitive for the detection of differences in nutrient supply of annatto (*Bixa orellana*) in central Amazonia.

	Sampling date	Leaf class
N	no recommendation possible	
P	January	medium – old
K	no recommendation possible	
Ca	August	new
Mg	any time	medium

palm, *Pueraria* and grass in the same experiment, the highest faunal densities and a relatively high small-scale diversity of invertebrate families were found in the annatto litter (Vohland & Schroth, 1999), suggesting that this abundant, high-quality litter also affected the decomposer community (and thus nutrient cycling) as well as other fauna living in the litter. Annatto, therefore, is a valuable component of mixed tree crop systems and is especially suited to associations with crops that produce litter of low quality and nutrient contents such as cupuaçu, which is cultivated widely in the region.

In this experiment, annatto yields were low and decreased after 1998, probably as a result of infertile soil and increased shading by the Brazil nut trees. Annatto is considered shade sensitive (Rosalen *et al.*, 1991), and the highest yields are obtained with full sun exposure (Castro *et al.*, 1994). This suggests that the species is best associated with small trees, such as cupuaçu, coffee (*Coffea* sp.) or *Citrus* spp., or with annual crops. In multi-strata systems with large, dominant trees such as Brazil nut, annatto is probably best grown at the plot boundary or in other open places, although this may restrict its potentially favourable influence on nutrient cycles in other parts of the plot.

There may have been a further reason for the low and decreasing annatto yields, however. At the end of the study period, it was noted that the trees had a lot of dead trunk wood and old branches from which no young branches had re-sprouted after the post-harvest pruning. This could indicate that the drastic annual pruning had progressively weakened the plants and reduced their ability to re-sprout and, therefore, to set fruit. As the flowers and fruits of annatto are formed at the extremities of new branches, regular pruning is recommended to increase fruit set. There is, however, ambiguity concerning the optimal intensity and frequency of pruning (Rosalen *et al.*, 1991). Falesi and Kato (1992) suggested that drastic pruning of adult annatto trees favours the production of seeds, but stressed the preliminary nature of this recommendation. Castro *et al.* (1994) recommended annual or biannual pruning to remove diseased and poorly formed branches (i.e., more selective pruning than that applied in the experiment reported here) and to reduce the height of the plant. Removal of large parts of the crown can negatively affect the root system of trees such as tea (*Camellia sinensis*) (Kandiah *et al.*, 1984) because of the shortage in photosynthates, and it seems possible that such effects also occurred with annatto in this experiment. The drastic post-harvest pruning was also a major drain on the nutrient reserves of the trees (Table 5). These arguments suggest that in future experiments with annatto, either biannual pruning or more selective annual pruning than that used in the present experiment should be considered.

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