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Isotopic evidence of the transfer of nitrogen fixed by legumes to coffee trees

Didier Snoeck [(1)], Federico Zapata [(2)], Anne-Marie Domenach [(3)]

[(1)] CIRAD–CP. BP 5035. 34032 Montpellier cedex 1. France. E-mail : snoeck@cirad.fr.
[(2)] IAEA. Joint FAO/IAEA Division. Vienna. Austria.
[(3)] UMR 5557. Université de Lyon 1. France.

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The use of isotopic methods has made it possible to quantify the contribution of nitrogen fixed by a legume crop grown in a coffee plantation. Thanks to the use of the natural ^{15}N abundance assessment technique, we were able to show that, in field condition, roughly 30% of the nitrogen effectively fixed by a legume (including biomass, roots and root exudates) were transferred to the associated coffee trees. The quantities of N transferred by legume prunings to sole coffee trees were measured to evaluate the amount of N transferred to coffee *via* litter fall or soil.
Keywords. *Coffea arabica*, *Desmodium*, *Leucaena*, N-15, isotope dilution, Burundi.

Mise en évidence du transfert de l'azote fixé par des légumineuses aux caféiers. L'utilisation des méthodes isotopiques a rendu possible la quantification de la contribution de l'azote fixé par une légumineuse cultivée dans une plantation de caféiers. Grâce à l'utilisation de la méthode des abondances isotopiques naturelles de l'azote, nous avons pu démontrer que, dans des conditions de cultures *in situ*, environ 30 % de l'azote atmosphérique effectivement fixé par la légumineuse (biomasse, racines et exsudats racinaires) sont transférés aux caféiers associés. Les quantités de N transférées aux caféiers par les émondes de légumineuses appliqués comme paillis ont été mesurées pour évaluer l'importance du transfert de N au caféier *via* la litière ou le sol.
Mots-clés. *Coffea arabica*, *Desmodium*, *Leucaena*, N-15, dilution isotopique, Burundi.

1. INTRODUCTION

Mulching is mainly done in coffee plantations to introduce organic matter and nutrients but also to protect the soil from erosion and evaporation. It is practised by applying, around the coffee trees, grasses or crop residues recuperated from the adjacent fallows. However, this method is labour demanding and results in a decline of soil fertility in the fallowed fields and induces a subsequent decrease in the productivity of annual crops after some years. These constraints have led to the use of intercropping legume inside the coffee plantation. Furthermore, legume crops have also the advantage to introduce atmospheric nitrogen, which is an important element for the nutrition of coffee trees. In order to make the association efficient, the legume should fix atmospheric nitrogen and transfer part of it to the associated plant.

Observations of N increase in coffee trees thanks to cultural association with legume crops have been reported by various researchers. For example, in Congo, an increase of 22% of total nitrogen in coffee leaves has been found when associated with *Leucaena leucocephala* (Lam.) De Wit cultivated as a cover crop (Snoeck, 1961). In Venezuela, Aranguren *et al*. (1982) observed that *Erythrina* or *Inga* trees planted inside a coffee plantation could compensate the N exportations due the parchment coffee (pulps of the cherries were returned to the field). In Costa Rica, Alpizar *et al*. (1985) as well as Glover and Beer (1986) observed that the N concentration in the coffee leaves was slightly higher when associated with *Erythrina* than with *Cordia.* In the same country but in other trials, Babbar and Zak (1994) observed a better soil N mineralization (15 g of N m^{-2} $year^{-1}$) in coffee cultivated under *Erythrina* than in the single coffee plantation (11 g of N m^{-2} $year^{-1}$).

The objective of this study is to quantify the main N cycling processes occurring in an associated coffee–legumes system by means of ^{15}N isotope techniques. For this purpose:

1. Leaf samples were taken in plantations where coffee trees are cultivated with legume cover crops to quantify the percentage of N_2 fixed by a legume and its possible transfer to coffee trees. The method

using natural ^{15}N abundance was used to estimate these percentages.
2. The percentage of N transferred by sole legume prunings to coffee trees were calculated to differentiate the share of N taken by coffee plants *via* litter fall or soil. The ^{15}N enrichment method was used for this experiment.

2. MATERIALS AND METHODS

2.1. Global N transfer in some coffee–legume associations

The objective was to measure the quantities of nitrogen fixed by legumes and actually transferred to coffee trees. Observations were realized in some coffee plantations using legumes either as cover crops or as shade trees in two different sites (Kayanza and Rukoba).

Description of sites. The Kayanza site, situated in the Buyenzi region (2.7° South; 29.6° East), is characterized by an equatorial climate altitude (1,700 m above see level). The rainy season runs from October to May with a mean annual rainfall of 1300 mm; a daily average temperature of 19°C all year round and a daily thermal amplitude of 10°C. Soils are classified as ferralsol and are moderately saturated in mineral nutrients and moderately acidic (pH_{H_2O} = 5.5).

The Rukoba site, situated in the Kirimiro region (3.1° South; 30.1° East), is characterized by a daily average temperature equal to that of Kayanza, but the rainfall is lower (1240 mm). Soils are also classified as ferralsol but they are very unsaturated and acidic (pH_{H_2O} = 4.5).

The trials were planted in the fields in December 1990 at Kayanza and in December 1991 at Rukoba. Soil and leaf samples were taken in April 1994; i.e. after a period of two years (Kayanza) and one year (Rukoba) of biological N fixation (BNF) and transfer.

Experimental designs. All trials were planted in randomized blocks with six replications. In each block, coffee trees were planted spaced 2.5 m × 1.5 m (i.e. 2,666 plants per hectare). The coffee tree species was the *Coffea arabica* (L.) var. Bourbon cultivar Jackson 2; its mature average height is between 2.5 and 3.0 m.

In trials using legume crops as cover crops, the species used were *Flemingia macrophylla* (Willd.) Merr., *Desmodium intortum* (Mill.) Urb. and *Leucaena leucocephala* (Lam.) De Wit. These legumes were planted in double hedgerows in the middle of coffee tree alleys. Inside a double hedgerow, the plants were spaced 30 cm × 30 cm apart in quincunx (i.e. 26,666 plants·ha^{-1}). Each trial compares the four cultural association types to a control composed of single coffee trees.

In trials using shade tree legumes, the species used were *Leucaena diversifolia* (Schlecht.) Bentham, *Calliandra calothyrsus* (Meissn.) and *Erythrina abyssinica* (Lam.). The shade trees were spaced 10 m × 10 m (i.e. 100 trees·ha^{-1}). The elementary plot was then constituted of a block of 24 coffee trees (4 × 6) with a shade tree in its centre.

Observations and measures. In the plantations aged two years (Rukoba) or four years (Kayanza), leaf samples were taken on the legumes, and on coffee trees being adjacent to legumes or distant from them. The coffee tree near a legume was chosen sufficiently close to benefit from the fixed nitrogen due to the cohabitation of both root systems and to the litter of the legume crop, while the distant coffee tree was chosen in order to have no contact with the legume roots or with its litter.

The samples were then oven-dried, ground into fine powder and thereafter analysed for total N% and % ^{15}N a.e. (atom excess). The percentages of total N in plants and in the soil were measured by the Kjeldahl method. The percentages of ^{15}N a.e. were measured with a mass spectrometer Finnigan type ΔE (Bremen, Germany) coupled with an elemental analyser (SCA, CNRS, Vernaison, France) whose precision is ± 0.3 ‰.

Analysis of the percentage of nitrogen fixed by the natural ^{15}N abundance method. The percentage of atmospheric nitrogen fixed by legumes and the percentage of nitrogen transferred to coffee trees were calculated following measurements of natural ^{15}N abundance in legume crops and in coffee trees associated or not to legumes. This method is based on the observations that:

1 two stable isotopes of nitrogen (^{14}N and ^{15}N) naturally exist and
2 the ^{15}N atoms ratio in the air is constant (0.3663%) while it varies in the soil from 0.3630% to 0.3730% ^{15}N atoms (Amarger *et al.*, 1979; Bardin *et al.*, 1977; Ledgard *et al.*, 1985; Shearer *et al.*, 1974).

Considering these differences, a legume that takes a part of its needs from the N derived from atmosphere (Ndfa) through fixation and the rest from the N derived from soil (Ndfs) will have an isotopic percentage ($\%^{15}N_{leg.}$) situated between the isotopic ratio of the soil ($\%^{15}N_{soil}$) and the isotopic ratio of the atmosphere because of the biological N fixation (BNF). This value is corrected by a factor taking in account the isotopic fractionation occurring during N_2 fixation ($\%^{15}N_{fixation}$). The percentage of nitrogen introduced in the system by the N-fixing plant is calculated by the isotope dilution equation (Bardin *et al.*, 1977; Fried, Middleboe, 1977):

$$\text{Ndfa\%} = \frac{\left(\%^{15}N_{soil} - \%^{15}N_{leg.}\right)}{\left(\%^{15}N_{soil} - \%^{15}N_{fixation.}\right)} \times 100.$$

Due to the very small differences between the ^{15}N natural abundance in the air and in the soil, data are often expressed in thousandths where the % of ^{15}N in the air usually serves as the reference. One then uses the

$$\delta^{15}N = \frac{\left(\%^{15}N_{sample} - \%^{15}N_{air}\right)}{\left(\%^{15}N_{air}\right)} \times 1000$$

By convention, the $\delta^{15}N_{air} = 0$ ‰.

The values of fixation ($\delta^{15}N_{fixation}$) for the legumes used were obtained by cultivating nodulated N-fixing plant in N-free media so that the nitrogen taken up by the legume can only come from the atmosphere. (column "fixation" in **table 1**). The $\delta^{15}N_{ref.Pl.}$ measured from a reference plant (non-leguminous) fully dependant of soil N is often used in place of the $\delta^{15}N_{soil}$.

Assessment of nitrogen transfer from legume to coffee trees. Some coffee trees were planted close to the legumes. As a consequence, they received their litter and had their root systems mixed with those of the legumes. While other coffee trees were planted far from the legume and did not benefit from the association. Due to this planting design, it was possible to calculate the percentage of nitrogen introduced by N_2 fixation in the cultural system by means of the ^{15}N excess measured in leaves sampled on each of the two associated plants and on the distant coffee tree. It has been shown that, when a non N-fixing plant was cultivated in association with a N-fixing plant, nitrogen taken up by the non N-fixing plant in the soil is derived from two sources: X% coming from the N_2 fixation by the legume and Y% coming directly from the soil N (Kurdali *et al.*, 1990). This can be expressed by a system of two equations with two unknown quantities:

$$X\% \times \delta^{15}N_{fixation} + Y\% \times \delta^{15}N_{ref.Pl} = 100\% \times \delta^{15}N_{assoc.}$$

$$X\% + Y\% = 100\%$$

where the $^{15}N_{fixation}$ was obtained from the N-fixing plant cultivated in a completely N-free medium, $^{15}N_{ref.\ Pl.}$ was obtained from the coffee plant cultivated sufficiently far from the legume to be out of its root area, while $^{15}N_{assoc.}$ was obtained by the coffee plant cultivated close to the legume. The resolution of this system gives the percentage of nitrogen (X%) provided to the association by the N fixation. The following formula is obtained:

$$X\% = \frac{\delta^{15}N_{ref.pl.} - \delta^{15}N_{assoc.}}{\delta^{15}N_{ref.pl.} - \delta^{15}N_{fixation}} \times 100.$$

The **figure 1** gives an illustration of the process.

Table 1. $\delta^{15}N$ observed in legume crops and coffee trees from the isotopic value of leaf samples — *$\delta^{15}N$ observé dans les légumineuses et les caféiers à partir des valeurs isotopiques d'échantillons foliaires.*

Type of legumes in association		**Legume**		**Coffee tree leaves**	
		fixation (‰)	**legume** (‰)	**association** (‰) (adjacent to legume)	**ref. plant** (‰) (distant from legume)
Rukoba					
Flemingia macrophylla	(i)	- 1.20	3.85 ± 0.6	7.55 ± 0.5	8.12 ± 0.4
Leucaena diversifolia	(i)	- 1.90	4.14 ± 2.0	7.63 ± 0.6	8.12 ± 0.4
Kayanza					
Flemingia macrophylla	(i)	- 1.20	2.86 ± 0.4	3.38 ± 0.2	3.85 ± 0.3
Desmodium intortum	(i)	- 1.00	1.30 ± 0.5	3.60 ± 0.3	4.50 ± 0.3
Leucaena leucocephala	(i)	- 1.90	- 0.40 ± 0.3	1.20 ± 0.2	2.10 ± 0.2
Leucaena leucocephala	(i)	- 1.90	2.65 ± 0.5	2.94 ± 0.2	2.83 ± 0.3
Leucaena diversifolia	(s)	- 1.90	1.46 ± 0.5	4.55 ± 0.2	5.48 ± 0.3
Calliandra calothyrsus	(s)	- 1.42	3.82 ± 0.2	5.13 ± 0.1	4.57 ± 0.6
Erythrina abyssinica	(s)	- 1.15	3.17 ± 0.3	4.32 ± 0.4	3.38 ± 0.3

(i) legume crop as intercrop; (s) legume as shade tree.

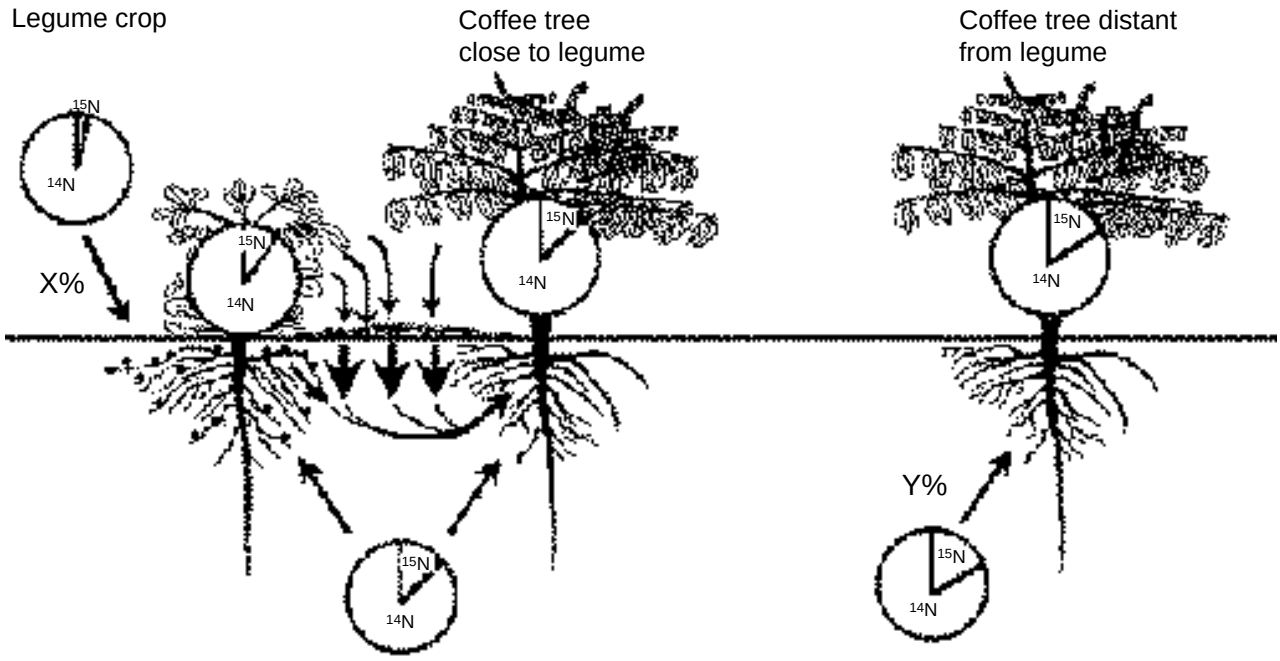

Figure 1. Evolution of ^{15}N in the soil and in the plants in the course of time — *Évolution du ^{15}N dans le sol et dans les plantes au cours du temps*.

2.2. N transfer by sole legume prunings to coffee trees

The objective of this trial was to calculate the amount of N in legume pruning of *Leucaena leucocephala* and *Desmodium intortum* transferred *via* the soil to one year old coffee trees.

Experimental design. The experiment was arranged in a complete randomized block system comparing three treatments replicated four times. The experimental treatments were the following:

1. No mulch or N fertilizer (control),
2. 3 kg of fresh-labelled prunings of *L. leucocephala.* i.e.: 28.5 g N at 0.1157%^{15}N a.e.,
3. 3 kg of fresh-labelled prunings of *D. intortum*. i.e.: 18.1 g N at 0.5117%^{15}N a.e.

The legume prunings were applied at the base of one year-old coffee trees and left in the field for one year. The soil and climate conditions are corresponding to the Rukoba conditions.

Production of ^{15}N labelled prunings. To produce enough ^{15}N labelled biomass to mulch four coffee trees (i.e. ±3 kg of green leaves plus twigs), 40 plants of *Leucaena* and five plants of *Desmodium* were grown for six months in two separate plots (lined with plastic to prevent losses). The legumes were not inoculated with rhizobia to avoid ^{15}N dilution by N_2 fixation. Plants were watered with a solution of ^{15}N labelled urea, i.e. *Leucaena* received 1% ^{15}N a.e. labelled urea and *Desmodium* received 2% ^{15}N a.e. labelled urea. For this purpose, 12 g of N^{-15} labelled area (with 1% or 2%^{15}N a.e. according to the plant) were dissolved in a 12 litres watering can. Then, 1.2 litres were applied per plot and per application at the frequency of two applications per week during 25 weeks. After six months, the legume plants were cut and all prunings were collected.

After one year, labelled *Leucaena* and *Desmodium* prunings, coffee leaves and soil samples were taken from each of the treatments and %^{15}N a.e. was determined (**Table 2**).

Table 2. Effect of two legume mulches enriched in ^{15}N on the % ^{15}N a.e. of soil and coffee tree leaves — *Effet de deux litières de légumineuses enrichies en ^{15}N sur le % d'exès en ^{15}N du sol et des feuilles de caféier*.

Treatments	%^{15}N a.e.		% Ndff
	Coffee	Soil	in Coffee
Control	0.0024	0.0029	
Leucaena	0.0171	0.0072	12.7
Desmodium	0.0451	0.0155	8.4

Quantification of N uptake by coffee plants. In practice, when a nitrogen source is applied to a plant, only part of the nitrogen provided by the source is taken up; the rest is immobilized in the soil or lost from the soil / plant system. Therefore, the percentage of N in the plant derived from a labelled fertilizer (%Ndff) is directly related to the ratio between quantities of ^{15}N isotopes in the plant and in the applied nutrient. This is expressed by the formula:

$$\% \text{ Ndff} = (E_{pl}/E_F) \times 100$$

where E_{pl} is the $\%^{15}N$ a.e. measured in the plant and E_F is the %15N a.e. measured in the fertilizer.

Due to the light labelling used, the natural ^{15}N abundance in the plant or soil cannot be neglected and the actual isotopic excess in the plant (E_{pl}) is calculated by the difference between the $\%^{15}N$ a.e. measured in the sample receiving the treatment and the $\%^{15}N$ a.e. measured in the same part of the untreated reference sample (control treatment):

$$E_{pl} = (\%^{15}N_{treatment} - \%^{15}N_{control}).$$

3. RESULTS AND DISCUSSION

Table 1 presents the $\delta^{15}N$ measured in the legume crops, in coffee trees adjacent to legumes (column "association") or distant from them (column "ref. plant"). Isotopic ratios in both N-fixing and non N-fixing plants are higher in the Rukoba site than in the Kayanza site. An important variation can be observed between adjacent fields in a same location even though the same crop is used (e.g. the two *L. leucocephala*). This horizontal variation was also observed by Bremer and Van Kessel (1990) who found an horizontal variation of 6 δ units over 42 m in the soil. The comparison of data in the associated or the reference plant shows the importance to have the reference plant close to the legume plant to estimate the %Ndfa without introducing a bias due to soil variation. We verified that, in a same location and a same trial, chemical analyses (base elements and CEC) of the soil under sole coffee trees and coffee trees associated to legumes did not show significant differences; so that the differences observed in the $\delta^{15}N$ measurements can be attributable mainly to the BNF.

Another limit usually ascribed to the natural ^{15}N abundance method is the influence of ^{15}N concentration in the soil over the accuracy of the data. The values observed in our survey reveal that, fortunately, the natural ^{15}N abundance levels are sufficiently high and uniform to ensure reliable N_2 fixation measurements in the case of Burundi coffee plantations. When N_2 fixation capacities of the legumes are good, associated coffee trees present isotopic values lower than farther away coffee trees; thus indicating that the coffee trees effectively take benefit from atmospheric N *via* the litter and roots of the N-fixing plant.

The N% derived from atmosphere as fixed by legume crops (%Ndfa) and N% transferred to the associated plants via litter and soil (X%) are given in **table 3**. The percentage of nitrogen in the associated coffee tree coming from the N_2-fixation by the legume is directly proportional to its fixation capability.

With legumes cultivated in hedgerows in the plantation, coffee trees can benefit from the nitrogen fixed by the legume in quite noticeable proportions (6% to 22% at Kayanza). If the N-fixation exists but is low, (in the range of 20%), there is very little to no transfer to the association. We cannot assume that there will be any competition between the associated plants, but the farmer will not be interested in the intercropping method.

When the BNF is too low, the nitrogen fixed by the legume is not sufficient to satisfy both crops and deficiency symptoms can be observed in the field. This highlights the importance of using cultural techniques adapted to the association.

With legumes cultivated as shade trees, associated coffee trees did not get much of the nitrogen fixed by the legume tree. We observed a little transfer when %Ndfa is high (48% for *L. diversifolia*) to no transfer of the N fixed at all when %Ndfa is low (less 21% for *Calliandra* or *Erythrina*). An explanation can be found in the difference between the number of plants used in

Table 3. % Ndfa in the legume crops, X% and relative %N transferred to coffee trees — *% de N_2 fixé par les légumineuses, X % et % N transférés aux caféiers*.

Type of association		Ndfa (%)	X% (%)	%N transferred (in % from Ndfa)
Rukoba				
Flemingia macrophylla	(i)	42 ± 5	6 ± 3	14.3 ± 2
Leucaena diversifolia	(i)	39 ± 19	5 ± 4	12.8 ± 4
Kayanza				
Flemingia macrophylla	(i)	20 ± 9	6 ± 4	30.0 ± 4
Desmodium intortum	(i)	50 ± 5	16 ± 2	32.0 ± 3
Leucaena leucocephala	(i)	52 ± 4	22 ± 1	42.3 ± 1
Leucaena leucocephala	(i)	0	0	
Leucaena diversifolia	(s)	48 ± 5	15 ± 4	31.3 ± 3
Calliandra calothyrsus	(s)	20 ± 13	0	
Erythrina abyssinica	(s)	21 ± 4	0	

(i) legume crop as intercrop; (s) legume as shade tree.

each case. In trials where legumes were planted in hedgerows, each coffee tree was surrounded by two rows of legumes (*Leucaena*, *Desmodium* or *Flemingia* in our trials): one row uphill plus one downhill. This makes 10 plants per coffee tree (26,666 plants·ha^{-1}). While in trials where legumes were cultivated as shade trees, there was only one plant every 10 m corresponding to only 100 plants·ha^{-1} or 1 legume tree per 26 coffee trees.

The N% transferred to the coffee trees in relation to the amount of N_2 fixed by the different legume crops is calculated by the ratio between the N in the coffee tree coming from the N_2 fixation by the N fixed by the legume: $(X\%_{coffee}/Ndfa_{legume})\times100$. The ratio is quite constant for the various plots in a same location; i.e. more or less 13% at Rukoba and from 30% at Kayanza and up to 42% in this location for trials where the association was very effective (**table 3**). The differences between the two locations can be attributed to various factors; out of which the difference between the age of the trials (one year fixation in Rukoba, two years fixation in Kayanza) and also the difference between the soil fertility in the two locations (soils are more fertile in Kayanza than in Rukoba).

The N_2 fixed by the legume crop is put into the cultural association through two complementary modes: the litter fall and the cohabitation of the root systems (*via* the root exudates, dead nodules, etc.). In our trials, around 8 to 12% of the N is brought through the sole litter applied as mulch (**table 2**), while 15 to 20% of N is transferred yearly to the associated coffee trees when the whole legume is grown inside the coffee plantation. The comparison of the trials indicates that approximately 75% of N_2 is transferred through the litter fall and the remainder 25% is transferred through the soil.

Thanks to the use of natural ^{15}N abundance techniques, we could show the relationship between cultural practices, BNF capabilities of some legume crops and N transfer to associated coffee trees. The use of this technique as a tool in trials could lead to a better understanding of the plantation conditions in relation with the cultural techniques; thus, help us to improve the use of legume intercrops as a mean of plantation sustainability.

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