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Cocoa (*Theobroma cacao* L.) trees benefit from N derived from the atmosphere when intercropped with the legume tree *Gliricidia sepium* (Jacq., Kunth ex Walp.)

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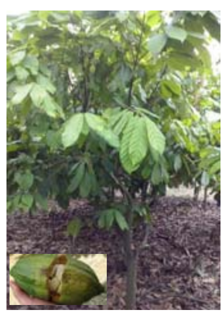
BACKGROUND

- Ghana is second largest producer and exporter of cocoa after the Ivory Coast (ICCO, 2013).



- In Ghana, cocoa is considered as the backbone of the economy, serving as source of foreign exchange and contributing significantly to the growth of the gross domestic product (David, 2013).

- In Ghana, intercropping shade trees with cocoa is a common practice among farmers and about 41 % to 58 % of cocoa farms use shade trees (Gockowski and Sonwa, 2007).

Soil and climate

- Cocoa trees require deep soil (coarse particles to a depth of 1.5 m) with good drainage, and soil pH between 5.0 and 6.5
- Cocoa is tropical crop that needs warm and humid climate, with minimum temperature of 18 and maximum temperatures of 32 ^{o}C (Asare and David, 2010).
- It is highly susceptible to drought and requires rainfall of 1,500-3,000 mm/year
- There are two cocoa growing seasons in Ghana:
  - the major rainy season starts from March to July
  - the minor season starts from late August to November.

The problem

- However, the problem of low N fertility (Bos *et al.,* 2007; IFDC, 2012) of soils in cocoa farms have caused yield to decline (450kg beans/ha, compared to normal yields of approx. 800kg/ha).
- This is because farmers at the early stages of cocoa production could clear virgin forest for cocoa cultivation, but due to limited access to land, there has been continuous cultivation of cocoa on lands with little or no nutrient replenisment (Baah, 2011).
- The introduction of inorganic N-based fertilizers has achieved little success especially due 1) their cost and 2) to the fact that they are poorly accessible in remote areas (Opoku-Ameyaw *et al.*, 2012)
- Currently, only **20% of the 1.6million ha** under cocoa cultivation are receiving fertilizer application and only **17%** of cocoa farmers in Ghana apply any type of inorganic fertilizers (IFDC, 2012)

Possible Solution ?

- **Legume trees when intercropped with cocoa, besides providing shade, enrich the soil with N thanks to their ability to develop symbiosis with N_2-fixing rhizobia**

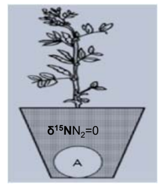
- This biological N is ´free´, cheap, unlimited and has no adverse effect on the environment (Tscharntke *et al.,* 2011)
- Economically, this BN could provides an affordable alternative to or supplement the inorganic fertilizer in cocoa plantations.
- Among legume trees, *Gliricidia sepium* is currently the most commonly planted tree to provide shade for cocoa trees in Ghana (Anim-Kwapong, 2003)

Does *Gliricidia sepium* (Jacq. Kunth ex Walp.) have the potential to fix N_2 in tropical soils of Ghana?

To answer the question, we used the ^{15}N natural abundance technique

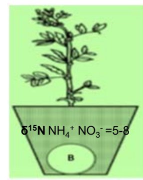
The technique compares delta (δ^{15})N of plants developing symbiosis with Rhizobia (gliricidia in my study), with δ^{15}N of one or more reference plant(s), that depend on soil N only (cocoa trees in my study).

$\delta^{15}N \sim 0$ ‰



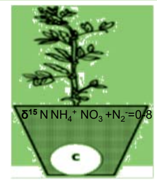
If the tree derives all its N from N_2-fixation

$\delta^{15}N \sim 5\text{-}8$ ‰



If the tree derives all its N from uptake of soil N sources

$\delta^{15}N$ between 0 and 8 ‰



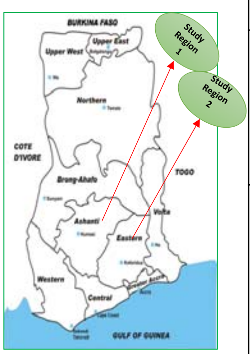
If the tree has two N sources (N_2 fixation and soil N)

Formula used to estimate the % N derived from the atmosphere (%Ndfa) (ACIAR, 2008 and Boddey *et al.*, 2000)

$$\%Ndfa = \frac{\delta^{15}N\,(reference) - \delta^{15}N\,(gliricidia)}{\delta^{15}N\,(reference) - \delta^{15}N\,(B\text{-}value)} \quad X\,100$$

The B-value is the δ^{15}N value of gliricidia when N_2 is the sole N source. The B-value used was -0.45 ‰

- 2 cocoa growing regions in Ghana were selected for study



Characteristics	Site 1	Site 2	Site 3	Site 4	Site 5
Type of intercropping	Gliricidia intercropped in rows with cocoa (25 % gliricidia and 65 % cocoa).	Gliricidia is sparingly intercropped with cocoa (15 % gliricidia and 70 % cocoa)	Gliricidia is sparingly intercropped with cocoa (68 % cocoa and 17 % gliricidia)	Gliricidia is sparingly intercropped with cocoa (18 % gliricidia and 62% cocoa).	Pure gliricidia and pure cocoa stands.
Nutrient management	No external nutrient supply	Inorganic N fertilizers, mainly Ca nitrate (approx. 12 kg N/ha) in the previous 5 years	Ca nitrate yearly applied (approx. 20 kg N/ha) since establishment	No external nutrient supply	No external nutrient supply

- The cocoa trees were planted at 3mx3m and gliricidia at 9mx9m
- The cocoa trees selected for sampling were at least 12 m from the closest gliricidia tree

Leaf sampling

- 3 sites were selected in the dry season and 5 sites in the rainy season (including the 3 in the dry season)
- fully expanded leaves of gliricidia and cocoa (as reference plant) trees (3 replicates per site) were sampled

- soil samples (at 0-30 and 30-45 cm depth) were also collected
- The sampled leaves and soils were oven dried, milled and analyzed for %N and delta ^{15}N using the isotopic ratio mass spectrometer (EA-Flash 2000 ThermoFisher Scientific instrument).

RESULTS

% N and $\delta^{15}N$ of soil samples (± s.e., Av. of seasons)

Site	N (% D.W)	$\delta^{15}N$ ($^{0}/_{00}$)
1	0.096± 0.002	7.35± 0.11
2	0.094±0.003	7.56± 0.10
3	0.187± 0.04	7.07± 0.08
4	0.090±0.001	6.92± 0.13
5	0.091± 0.002	6.93± 0.12

Result

Leaf δ^{15}N

(averages ± s.e.)

	δ^{15}N ($^{0}/_{00}$)		Significance
	Gliricidia	Cocoa	
Site 1[x]	3.13 ±0.04	4.30 ±0.02	*
Site 2[x]	2.75 ±0.06	4.24 ±0.15	*
Site 3[x]	3.85 ±0.06	3.66 ±0.07	n.s.
Site 4[y]	3.63 ±0.09	8.40 ±0.09	*
Site 5[y]	3.38 ±0.02	6.27 ±0.02	*

[x]Average of dry and rainy season

[y] Data refer only to rainy season

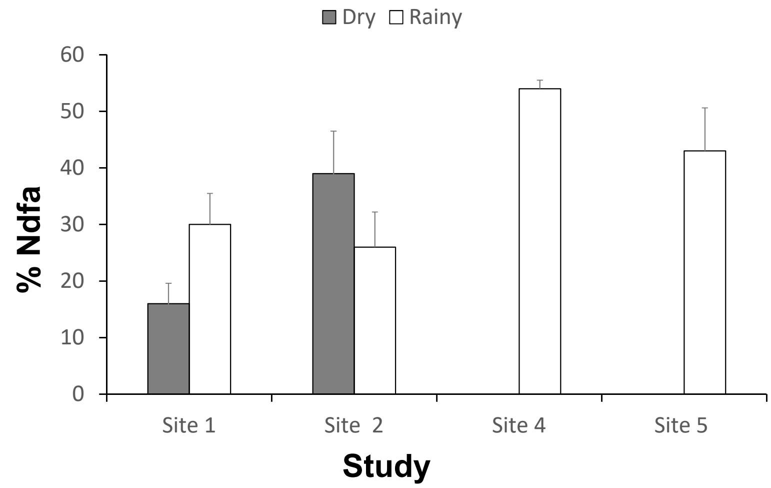
Result

N concentration (% DW), δ^{15}N of selected gliricidia organs and litter, cocoa leaves and orange leaves sampled from pure stands (site 5). Letters a to f indicate statistical difference ($P < 0.05$) among columns; averages ± s.e.

Species and organ	**N (%)**	**δ^{15}N ($^{0}/_{00}$)**
Gliricidia leaves	3.82± 0.04 a	3.38± 0.02 c
Cocoa leaves	2.81± 0.03 b	6.27± 0.02 a
Orange leaves	1.90±0.02 d	4.941±0.05 b
Gliricidia litter	2.61± 0.02 c	3.59± 0.03 c
Gliricidia shoot axes	1.46± 0.01 e	2.31± 0.07 d
Gliricidia roots	0.99± 0.01 f	2.18± 0.10 d

N derived from the atmosphere (Ndfa)

- The Ndfa recovered in gliricidia leaves ranged from 22 to 55 % of total leaf N



Result

Estimated amount of N and annual aboveground growth of gliricidiia (Kg DW/tree) averages ± s.e.

Plant diameter (cm)	N concentration (%)		Total Leaf biomass kg (DW)/ tree	Total shoot axis biomass Kg(DW)/tree	Amount of N (kg/tree)	
	leaves	Shoot axis			Leaves	shoot axes
19.8±0.9	4.19 ±0.07	1.34 ±0.01	4.48±0.9	32.74±2.4	0.19	0.44

- we estimated that the annual above ground growth of gliricidia (shoots) contains 130-380 g atmospheric N (30% in leaves, 70 % in shoot axis).

Which rhizobia is (are) responsible for N_2 fixation in *Gliricidia sepium* ?

Root nodules were analysed by the microbiological team at Uni-Pisa – Prof. M. Giovannetti

- Gliricidia roots were sampled for root nodule at the end of the dry (January, 2016) and rainy (September, 2016) seasons.
- nodules were found only at the end of the rainy season.

- **Results revealed the presence of *Rhizobium tropici* and *Rhizobium etli* in all the four sites.**

If the gliricidia trees are pruned and the biomass applied as green manure under cocoa trees, how fast is its decomposition and N release?

To answer the question, the litterbag technique was used

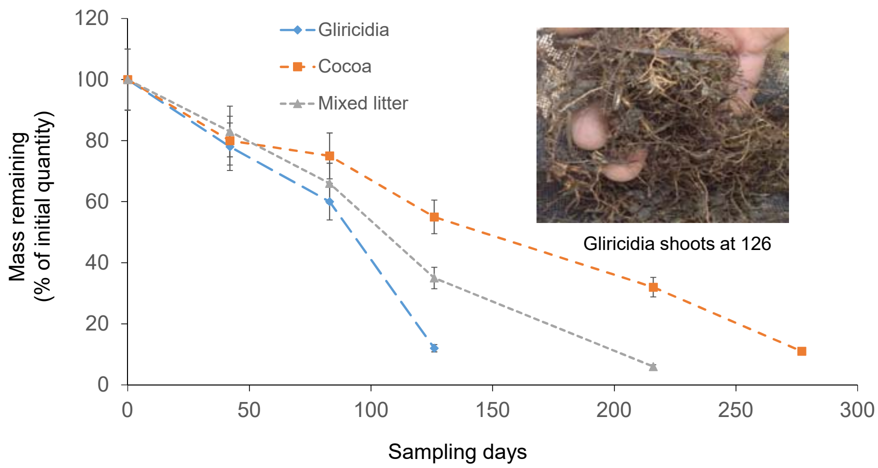
- three litter types were used:

(1) pure cocoa leaves litter
(2) pure gliricidia pruned shoots and
(3) a mixed litter (60 % cocoa litter and 40 % gliricidia pruning material).

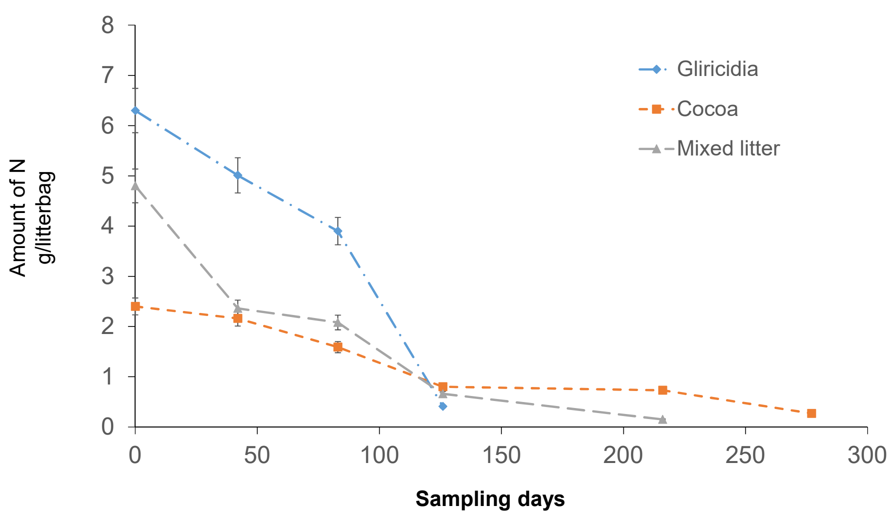
- A 1mm mesh nylon net was used to design a litterbag sewn into 30 cm x 25 cm.
- The equivalent of 150g (D.W) of each litter type was placed into each litterbag.
- A total of 9 cocoa trees (3 trees /litter type) were selected. Under each tree, we laid (on April, 2015) (at 0-15cm soil depth) seven litterbags of each litter type.
- sampling done at 43, 83, 126, 216 and 277 days.

RESULTS

Percent of initial biomass remaining during decomposition (bars indicate standard errors)



Pattern of Nitrogen release



Do cocoa trees close to gliricidia ones benefit from long term addition of gliricidia pruning material used as green manure?

To answer this quesion, we compared the leaf $\delta^{15}N$ of cocoa trees at different distances from gliricidia trees

- in all the four sites, we sampled leaves from two types of cocoa, depending on their distance from the nearest gliricidia tree:

1. Cocoa trees close (<5m) to the nearest gliriciida plant (´close cocoa´)
2. Cocoa trees distant (>12m) to the nearest gliricidia tree (´far cocoa´)

- we analysed the %N and $\delta^{15}N$ of the gliricidia leaves and the two cocoa types.

RESULT

$\delta^{15}N$ of gliricidia and cocoa leaves. Average (± s.e.) Letters a, b and c indicate statistical difference ($P < 0.05$) among means within rows.

Site	Gliricidia	Cocoa	
		Close	Far
1	*3.13±0.31 a*	**2.85±0.06 a**	*4.30±0.57 b*
2	*2.75±0.40 a*	**2.91±0.19 a**	*4.24±0.34 b*
3	*3.86±0.21 ns*	**3.20±0.50 ns**	*3.66±0.32 ns*
4*	*3.63±0.09 a*	**4.36±0.10 a**	*8.49±0.29 b*

- $\delta^{15}N$ of ´Close cocoa´ and Gliricidia were always, but in site 3, similar and , but the $\delta^{15}N$ of ´distant cocoa´ was significantly higher than both gliricidia and ´close cocoa´.
- cocoa close to gliricidia benefited from the N deriving from residues of the gliricidia tree due to their proximity to this legume tree, possibly thanks to the over time effect decomposition of its pruned biomass and N release.

GENERAL CONCLUSIONS (slide of 1/2)

❖Our estimates: 1 gliricidia tree= 130-380 g atmospheric N/ tree per year, thanks to the presence of *Rhizobium tropici* and *Rhizobium etli* in the root nodules.

❖The gliricidia shoots almost fully decomposed within 126 days (releasing about 95 % of initial amount of N)

❖ By comparing the leaf delta ^{15} N, we have proved that cocoa benefits from N present in gliricidia shoots when grown in proximity to the gliricidia tree in intercropping systems

❖ We speculate that if a single cocoa tree requires 30-70 g external N plant^{-1} for optimum growth and yields (Cocoa Research Centre, 2004; Van Vliet *et al*., 2015), then, intercropping systems with ratios gliricidia: cocoa from 1:2 to 1:12 should be enough to fulfill the N needs of cocoa without external N addition.

THANK YOU