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#### Key words:

Alley cropping; *Calliandra houstoniana*; green manure; *La Montaña*; *Mucuna pruriens*; organic fertilization; tropical deciduous forest

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# Agroecological and agroforestry strategies to improve organic hibiscus productivity in an Indigenous non-governmental organization from Mexico

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#### Abstract

La Montaña region, in southern Mexico, is characterized as a highly human-modified landscape with a rough topography, extreme poverty and structural violence. In this region, Xuajin Me'Phaa, an Indigenous non-governmental organization conformed by ca. 300 peasants, implements productive restoration projects and trades organic hibiscus (Hibiscus sabdariffa L.) as its main monetary income. Nonetheless currently, organic hibiscus yield is low compared to the potential yields in the region. Thus, it is necessary to explore alternative sustainable land management systems which enable farmers to increase hibiscus crop productivity, while halting land degradation. This study assessed the impact of six different agroecological fertilization protocols (AFPs) on hibiscus productivity planted in an alley cropping system with Calliandra houstoniana trees. The AFPs were based on the combination of three local plant amendments: C. houstoniana mulch, Mucuna pruiriens var. utilis green manure and hibiscus stover, and a commercial bio-fertilizer (Azospirillum + Rhizophagus). Simultaneously, the performance of C. houstoniana trees was assessed. The AFPs were applied in the alley cropping system and evaluated from 2016 to 2018. After 3 years, in the AFPs which included C. houstoniana mulch, hibiscus yielded significantly more  $(419 \pm 27 \text{ kg dry})$ calvxes ha<sup>-1</sup> in average) than AFPs which did not include this species  $(264 \pm 15 \text{ kg ha}^{-1})$ . The 18-month-old C. houstoniana trees yielded 0.6 t ha<sup>-1</sup> of dry biomass and 1.12 t ha<sup>-1</sup> of wooden stakes, a relatively low production. In conclusion, our results show that alley cropping with a denser arrangement of C. houstoniana trees in combination with mulching of this tree species, and use of mucuna green manure represent a promising agroforestry system for organic hibiscus production.

# Introduction

Excessive crop harvest removals, soil erosion and agriculture practiced in marginal areas, which are the most common causes of land degradation, deplete soil organic matter and nutrients, and eventually, lead to the loss of soil fertility (Sanchez and Leakey, 1997; Lal, 2001; Feller *et al.*, 2012; Panagos *et al.*, 2018; Nasir-Ahmad *et al.*, 2020). The loss of soil fertility affects farmers directly through the diminishing of the yield potential of their lands and ultimately raise concerns about food security at familiar, regional and global scale (FAO, 2015; Panagos *et al.*, 2018). Thus, it is of major importance promoting the adoption of economic and ecologically viable management practices to increase soil fertility and productivity.

Agroforestry maintains and enhances the long-term soil productivity by incorporating trees to arable lands. The trees can improve soil physical, chemical and biological properties by adding significant amount of above and belowground organic matter as well as releasing and recycling nutrients (Nair, 1993; Nair *et al.*, 2008; Jose, 2009). At the same time, agroforestry is aiming to diversify and sustain production to achieve social, economic and environmental benefits (Sanchez, 1995; Nair, 2013; Atangana *et al.*, 2014; FAO, 2017). The alley cropping systems are an important form of agroforestry practiced around the world, especially in the tropics and the sub-tropics (Nair, 2013; Silva-Galicia *et al.*, 2021). These systems combine lines of trees (or hedgerow contours, when implemented in steeplands) and crops in the space between such lines (i.e., the *alleys*, Kang, 1997; Nair *et al.*, 2008). Trees are pruned regularly to provide biomass to amend soil, either applied as mulch or incorporated into the soil. Fast-growing, N-fixing leguminous woody species, such as *Leucaena leucocephala* or *Gliricidia sepium*, are usually employed (Nair *et al.*, 2008; Nair, 2013).

The agroecological management of alley cropping systems may enhance the benefits provided by the perennial species. Organic amendments, like mulches and green manures, protect the soil, maintain adequate moisture and temperature, limit weed growth, and when they decompose, release nutrients and elements to constitute soil organic matter (Kang et al., 1981; Nair, 1985; Kang, 1993, 1997; Douthwaite et al., 2002). On the other hand, the lateral and deep root system of the woody perennials absorbs nutrients from deep soil layers and incorporates them into organic soil horizon by means of litter decomposition. Moreover, nitrogen fixed by bacterial symbionts in legumes can increase the soil nitrogen stocks (Palm, 1995; Kang, 1997; Rhoades, 1997; Sarvade et al., 2019). Tree roots also promote infiltration and mulch reduces soil runoff and the soil loss (Rhoades, 1997; Wei et al., 2007; Sarvade et al., 2019). Therefore, ecological services provided by alley cropping systems managed under agroecological practices, such as nutrient cycling, soil organic matter replenishment or the capacity to halt soil erosion, are of importance in degraded agricultural soils, especially in those areas where population has limited options to sustain their livelihood (Sarvade et al., 2019; Silva-Galicia et al., 2021).

La Montaña, a hilly region in the state of Guerrero (in southern Mexico) is characterized as a highly human-modified landscape with severe soil loss caused by practicing agriculture in sloping areas (>35%, Landa et al., 1997) and the predominance of small (<21 ha) and irregular forest remnants with disturbed vegetal communities (Borda-Niño et al., 2017). Indigenous groups inhabiting La Montaña region, Ñuu savi (known as Mixtec), Nahuas and Me'phaa (known as Tlapanecos), have been historically bordered to live in those ecologically fragile conditions (Galicia-Gallardo et al., 2019). Furthermore, their extreme poverty, inadequate access to basic services and structural violence exacerbate their ecological problems (Landa and Carabias, 2009; Galicia-Gallardo et al., 2021). All peasants in La Montaña practice subsistence agriculture and some of them cultivate hibiscus calyxes (Hibiscus sabdariffa, Malvaceae) (Galicia-Gallardo et al., 2019). Hibiscus calyxes are traditionally used in Mexican cuisine to prepare beverages, liquors and jams. Hibiscus cultivation is the most important economic activity in La Montaña and adjacent regions (SAGARPA, 2012).

*Xuajin Me'phaa*, an Indigenous non-governmental organization (INGO) located in Ayutla de los Libres, in the state of Guerrero, is integrated by ca. of 300 farmers from *La Montaña* who actively work in 'productive restoration' projects (Galicia-Gallardo *et al.*, 2019; Ceccon, 2020). Using agroforestry and agroecological techniques, 'productive restoration' seeks to recover some of the elements of the structure and function of the native ecosystem of the region and produce tangible products for local people while contributing to the connection of the landscape (Ceccon, 2013).

The income of the INGO comes from producing certified organic products, such as beans, fruits, coffee and honey; principal revenues, however, derive from selling organic hibiscus to an important supermarket chain (Galicia-Gallardo *et al.*, 2019, 2021). In a sustainability evaluation of two hibiscus cropping systems practiced in *La Montaña* (i.e., organic and conventional), it was found that organic production presents high scores in terms of independence from external inputs, cost/benefit relationship or social capital, nonetheless, the yields are much lower than the

optimal values (Galicia-Gallardo *et al.*, 2019). So, there is an urgent necessity to increase productivity in a sustainable way with agroecological fertilization options based on local inputs, like plant-based amendments. In this context, 'agroecological fertilization' refers to those management practices aiming to increase the crop plant productivity through improving the soil fertility (Havlin *et al.*, 2005; Abbot and Murphy, 2007; FAO, 2021).

In La Montaña, the traditional soil fertility management in organic hibiscus plantations includes various agroecological approaches without inputs of agrochemicals, superficially application of the hibiscus stover and minimum tillage. Some members of the INGO also include the nitrogen-fixing mucuna (*Mucuna pruriens* var. *utilis*, Fabaceae) as green manure (Galicia-Gallardo *et al.*, 2019). In the dry season, after the hibiscus cropping, plots are left as fallows with no soil cover, and occasionally, goats graze there (Galicia-Gallardo *et al.*, 2019). Finally, few members take care or plant woody native species and fruit trees in their plots (e.g., *Pinus* sp., *Quercus* sp., *Leucaena* sp., *Calliandra houstoniana, Mangifera indica, Psidium guajava, Citrus* sp.) (Galicia-Gallardo, 2015).

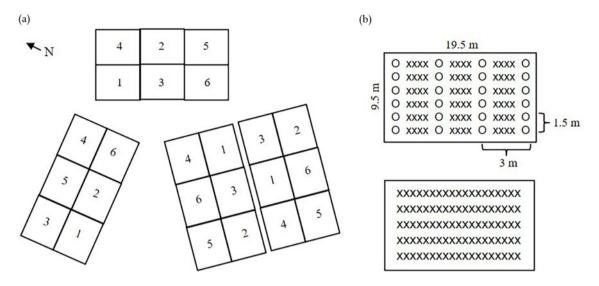
Silva-Galicia *et al.* (2021) evaluated the decomposition and nutrient release rates of the organic amendments traditionally employed by INGO members, and concluded that the combination of mulch of *C. houstoniana* tree (Fabaceae) and green manure of mucuna was the most promising soil amendment, because half of its biomass was lost to decomposition—and hence released nutrients—during the flowering of hibiscus crop. This mixture also provides elements to constitute soil organic matter. However, a practical evaluation of the effect of that mixture on hibiscus crop productivity under field conditions is still lacking.

Hibiscus crop production in La Montaña is at risk to diminish due to the ongoing processes of land degradation. Ultimately, such processes can compromise the future of the Xuajin Me'Phaa INGO and dozens of families who rely on the income generated. Also, the organic hibiscus trade generates higher revenues, and it is more sustainable than the conventional system (Galicia-Gallardo et al., 2019). Thus, here we investigated the performance of an alley cropping system with C. houstoniana trees (henceforth calliandra) and hibiscus crop during three hibiscus crop cycles (2016-2018). The soil in the alley cropping system was fertilized with agroecological fertilization protocols (AFPs), which refer to management practices which encompassed the application of organic amendments (mulch of calliandra, green manure of mucuna and/or hibiscus stover), as well as a commercial bio-fertilizer composed of mycorrhizas and diazotrophic bacteria. The impact of both, plant-based agroecological amendments and the bio-fertilizer, on the hibiscus yield was tested. In addition, the performance of the calliandra trees (expressed as biomass and woody stakes production) was assessed. The main hypothesis of the study was that AFP including calliandra and mucuna vegetal amendments as well as the biofertilizer would increase the hibiscus yield, because of the nutrients released and the physical protection provided by the amendments and the improved capacity to acquire nutrients associated to microorganisms in the bio-fertilizer.

# Methods

#### Study site

The study was conducted at an experimental plot of *Xuajin Me'Phaa* INGO, located in the foothills of *La Montaña* region, municipality of Ayutla de los Libres, state of Guerrero (southern



**Fig. 1.** Diagram of the experimental design in field. (A) Spatial distribution of the experiment. Blocks were subdivided into six plots, in which an agroecological fertilization protocol (AFP, indicated with numbers) was applied: *AFP1 'hibiscus stover'*; *AFP2 'hibiscus stover + bio-fertilizer'*; *AFP3 'hibiscus stover + mucuna'*; *AFP4 'hibiscus stover + calliandra + mucuna'*; *AFP5 'hibiscus stover + calliandra'*; *AFP6 'hibiscus stover + calliandra + bio-fertilizer'*. (B) Schematic design inside of the plots. At the top, a plot of alley cropping: calliandra trees ('O') among the hibiscus plants ('xxxx'). The alley cropping plots are indicated in 1A as 4, 5 and 6 received AFP which included mulch of calliandra. At the bottom, a plot of hibiscus in monoculture with numbers 1, 2 and 3 in 1A.

Mexico) (16°59'N, 99°05'W, 400 m above sea level). The climate is sub-humid with an annual precipitation of 1800 mm and the rainy season is from April to November (max. 434 mm in September), annual mean temperature is of 27.7°C (SMN, 2019). The soil was characterized as an *Umbric Stagnic Fluvisol* (*Episkeletic, clayic*) (WRB, 2007; Hernández-Muciño *et al.*, 2015). The vegetation was characterized by tropical deciduous forest with some patches of secondary vegetation and induced grasslands (Rzedowski, 2006).

# Study design and agroecological fertilization protocols

Four randomized blocks were arranged in an area of 3705 m<sup>2</sup> (926.3 m<sup>2</sup> block<sup>-1</sup>), every block was subdivided into six plots (Fig. 1A). AFPs (Table 1) consisted of the combination of three local amendments: hibiscus stover, mulch of calliandra and green manure of mucuna (carbon and nutrient concentration as well as insoluble fiber contents of these amendments are provided in Table 2). During the two first crop cycles (2016-2017), calliandra plants were too young to sustain the biomass production required for the alley cropping system. Even the mucuna planted in the plot presented a low biomass production. For this reason, the biomass of both species was brought from secondary forest patches in communities close to the study site (El Naranjo: 17°07'42"N, 99°02'43"W, elevation: 820 m.a.s.l. and Escalerilla Zapata: 17°7′51″N, 99°7′15″W, elevation: 580 m.a.s.l.). Mulch of calliandra was collected by pruning branches of trees, avoiding stems >3 mm diameter; the mulch obtained after this process showed an approximate proportion of 4:1 leaves/twigs; a larger proportion than that reported by Lehman et al. (1995) (1:5 leaves/twigs ratio). Green manure of mucuna consisted of chopped fresh leaves and twigs of <3 mm diameter in a natural 3:1 leaves/twigs ratio approximately. Finally, the hibiscus stover was gathered from the crop previously grown in the experimental plots and consisted of the air-dried hibiscus stems and capsules (the fruits without seeds) cut into pieces of 15-20 cm long and in a 3:1 stems/capsules ratio approximately

(the natural proportion after the harvest). Finally, for the third crop cycle (2018), the biomass imported from outside was complemented with calliandra and mucuna planted inside the plots. A mixture of bio-fertilizers based on *Rhizophagus irregularis* (*sin. Glomus intraradices*, an arbuscular mycorrhiza; MicorrizaFer<sup>®</sup> at  $1 \times 10^5$  spores kg<sup>-1</sup>) and *Azospirillum brasiliense* (free living, diazotrophic bacteria, Azofer<sup>®</sup> at  $5 \times 10^8$  cfu) was also included in the trial.

The AFPs were randomly assigned into the plots (Fig. 1A). Treatments without the calliandra mulch were applied in the hibiscus in monoculture plots (i.e., AFP 1, 2 and 3), whilst treatments including the mulch (i.e., AFP 4, 5 and 6) were exclusively applied in the alley cropping plots. The calliandra, mucuna and hibiscus stover vegetal amendments were applied superficially and spread uniformly on the soil plot. The total amount of calliandra was divided in two doses: The first application was made a week before sowing hibiscus seeds and the second, in mid-September. The bio-fertilizer was bought a couple of days before its application and the expiration day was verified directly with producers. It was applied as follows: seeds were sprayed with an adherent solution (prepared with carboxymethyl cellulose-an organic, non-toxic emulsifier-and non-colored water), uniformly covered with the bio-fertilizer and let them dry at room temperature for 3 h; after that, seed were sowed according to the traditional management (see below). In mid-September, the second dose was applied. In this occasion, the bio-fertilizer (1380 g of product) was dissolved in 200 Liters of non-colored water and sprayed directly to the hibiscus plants (to their basis and aerial parts). The experiment was carried out during a period of 3 years (2016-2018), according to the annual cycle of hibiscus crop (from late June to mid-December).

The cultivation of hibiscus was carried out according to the traditional management: 5-7 seeds per hole at  $50 \times 50$  cm spacing were sown round 26-28 June 2016 to 2018; the local seed variety (*criolla*) was employed. Weeds were removed by hand twice (in early August and mid-September), and agrochemicals were not employed. The harvest initiated by 11-13 December of each

**Table 1.** Composition of the agroecological fertilization protocols (AFP) with or without biofertilizers, green manure of fresh mucuna (*Mucuna pruriens*), hibiscus stover and mulch of air dried calliandra applied to the hibiscus (*Hibiscus sadariffa*) plantation in monoculture (HM) or in alley cropping with calliandra (*Calliandra houstoniana*) (AC)

Agroecological fertilization protocol (AFP)	Hibiscus stover 3 t $ha^{-1} yr^{-1}$	Green manure of fresh mucuna $5.3 \text{ t ha}^{-1} \text{ yr}^{-1}$	Mulch of air-dried calliandra 5.3 t $ha^{-1}yr^{-1}$	Bio-fertilizers
AFP1 (HM)	x			
AFP2 (HM)	X			x
AFP3 (HM)	X	X		
AFP4 (AC)	X	X	X	
AFP5 (AC)	X		X	
AFP6 (AC)	X		X	X

year. For sampling the hibiscus, the cropping area was subdivided in sampling plots of  $2 \times 2.5$  m each one, and samples were obtained from six of those subdivisions; thus, the total sampling area per treatment per plot was  $30 \text{ m}^2$ . Harvest was carried out according to the traditional management: Hibiscus calyxes were separated from the plant manually one by one and left to sun dry for 3 days until reaching 10% of humidity. The calyxes were weighted, and the yield was estimated as kg of dry calyxes per ha.

#### Performance of calliandra trees in the alley cropping system

Calliandra seeds were collected from January to March 2016 in neighboring forest patches in Escalerilla Zapata, sowed and maintained in nursery conditions for 4 months. In July 2016, seedlings >15 cm height were transplanted to the plots forming lines at  $1.5 \times 3$  m (density of 2222 trees ha<sup>-1</sup>) (Fig. 1B). A sample of randomly chosen trees (n = 75) was monitored monthly for 18 months (July 2016 to November 2017) to assess survival, height and diameter. Two years after (2018), trees were pruned twice at 70 cm high, the first by the end of July and the second one was at mid-October. Biomass (leaves and twigs) and woody parts were weighed separately to obtain the fresh and dry biomass.

# Statistical analyses

To analyze the hibiscus yield (kg of dry calyxes per ha) along the three production cycles (2016–2018), a generalized linear model with generalized estimating equations (Zeger *et al.*, 1988; Hardin, 2005) was carried out. This procedure is recommended for clustered data, as repeated measures (Hardin, 2005), e.g., measures obtained from observations in the same plot for consecutive cropping seasons. The 'AFPs' and 'production cycles' as well as the interaction 'production cycle–AFP' were analyzed as categorical variables. All statistical analyses were carried out in SPSS (IBM SPSS Statistics, vers. 23).

## Results

#### Hibiscus yield

Significant differences were found among the AFPs and the production cycles (years) (P < 0.05) but not for their interactions (P > 0.05) (Fig. 2). Considering the production cycles, yield was significantly higher in 2016 compared with 2017 and 2018. Comparing the yields under the AFPs, main differences were obtained for the treatments with calliandra mulch: *AFP4 'hibiscus* 

stover-calliandra-mucuna', AFP5 'hibiscus stover-calliandra' and AFP6 'hibiscus stover-calliandra-biofertilizer', which produced significant highest yield than non-mulched treatments. The differences were more evident in 2016, when those treatments yielded in average 637 ( $\pm$ 88 standard error), 610 ( $\pm$ 73) and 516 ( $\pm$ 57) kg ha<sup>-1</sup>, respectively. In 2017, however, the same AFPs yielded 318 ( $\pm$ 66), 272 ( $\pm$ 59) and 321 ( $\pm$ 45) kg ha<sup>-1</sup>, respectively; whilst in 2018, the same AFPs yielded 402 ( $\pm$ 30), 331 ( $\pm$ 17) and 365 ( $\pm$ 35) kg ha<sup>-1</sup>, respectively.

On the other hand, the AFPs which did not include the mulch of calliandra (*AFP1 'hibiscus stover'*, *AFP2 'hibiscus stover-biofertilizer'* and *AFP3 'hibiscus stover-mucuna'*) displayed general low yields. In the first production cycle (2016), those treatments yielded 345 ( $\pm$ 104), 313 ( $\pm$ 32) and 297 ( $\pm$ 1.5) kg ha<sup>-1</sup>, respectively. In 2017, yields were of 225 ( $\pm$ 60), 188 ( $\pm$ 31) and 272 ( $\pm$ 19) kg ha<sup>-1</sup>, respectively; and in 2018, the respective AFPs yielded 240 ( $\pm$ 27), 231 ( $\pm$ 10) and 280 ( $\pm$ 12) kg ha<sup>-1</sup>.

It is important to note that no significant productivity effect was detected with the biofertilizer, i.e., yield of *AFP2* '*hibiscus stover-biofertilizer*' was similar to the yield obtained with the *AFP1*, whilst yield of *AFP6* '*hibiscus* stover-calliandra-biofertilizer' was statistically similar to the other treatments with calliandra (*AFP4* and *AFP5*).

# Performance of calliandra trees in the alley cropping system

After 18 months of evaluation (July 2016 to November 2017), calliandra showed a survival rate of 87.7% (n = 75) and reached an average height of 120.9 (±30.2) cm and average diameter of 10.56 (±2.57) cm. After the first pruning (July 2018), calliandra showed a survival rate of 96.7% and produced 0.093 t of dry biomass ha<sup>-1</sup> in July and 0.52 t ha<sup>-1</sup> in October (0.613 t ha<sup>-1</sup> yr<sup>-1</sup> for total); as well as 0.603 and 0.518 t of dry wood stakes in the same months, respectively (1.12 t wood biomass ha<sup>-1</sup> yr<sup>-1</sup> for total) (Fig. 3).

## Discussion

## Hibiscus productivity under the AFPs

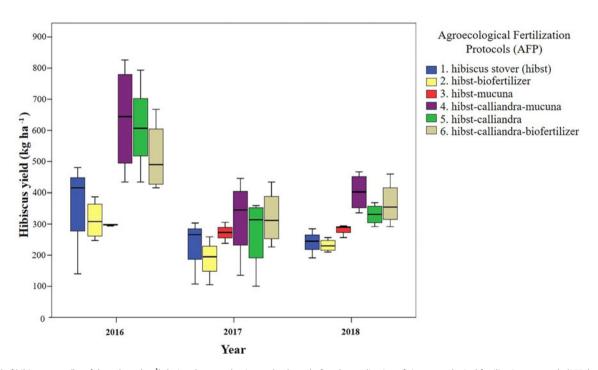
Tree species usually employed in alley cropping have 'high-quality litter', i.e., the chemical composition of their leaves contains >2% N, >0.2% P or <15% lignin (Vanlauwe *et al.*, 1997; Mafongoya *et al.*, 1998; Palm *et al.*, 2001). High-quality litter is associated with a relatively fast mulch decomposition, nearly complete nutrient release to the soil, as well as yield improving (Handayanto

Table 2. Average (±standar err	or) initial concentrations o	f carbon, nutrients and ins	oluble fibers in plant material

	Element/ratio						
Species	C (%)	N (%)	P (%)	C:N	C:P	N:P	
Calliandra houstoniana (calliandra)	44.82 (±1.28) a	1.66 (±0.01) b	0.16 (±0.02) b	26.92 (±0.91) b	291.04 (±43) b	10.73 (±1.25) a	
Mucuna pruriens (mucuna)	44.18 (±0.71) a	2.52 (±0.03) a	0.27 (±0.01) a	17.52 (±0.14) c	166.85 (±11) c	9.53 (±0.64) a	
Hibiscus sadariffa (hibiscus*)	42.97 (±0.35) a	0.33 (±0.03) c	0.05 (±0.01) c	135.1 (±15.4) a	1039 (±233) a	1.52 (±1.54) b	
Insolu	uble fibers						
	L (%)	Cell (%)	Hem (%)	L:N	Cell:N		
Calliandra houstoniana (calliandra)	17.51 (±0.37) a	20.54 (±0.56) c	10.04 (±1.9) c	10.51 (±0.3) b	12.33 (±0.28) b		
Mucuna pruriens (mucuna)	12.15 (±0.02) b	27.73 (±0.32) b	16.95 (±1.02) b	4.82 (±0.07) b	11.01 (±0.25) b		
Hibiscus sadariffa (hibiscus*)	11.82 (±0.42) b	45.88 (±1.12) a	24.16 (±7.3) a	36.84 (±2.7) a	144.67 (±18) a		

C, carbon; N, nitrogen; P, phosphorus; L, lignin; Cell, cellulose; Hem, hemicellulose.

Different letters within each parameter indicate significant differences. \*The plant material studied was stover. Modified from Silva-Galicia et al. (2021).



**Fig. 2.** Yield of hibiscus crop (kg of dry calyxes ha<sup>-1</sup>) during three production cycles (years) after the application of six agroecological fertilization protocols (AFPs). AFP 1, 'hibiscus stover'; AFP 2, 'hibiscus stover-biofertilizer'; AFP 3, 'hibiscus stover-mucuna'; AFP 4, 'hibiscus stover-calliandra-mucuna'; AFP 5, 'hibiscus stover-calliandra-biofertilizer'.

*et al.*, 1994; Myers *et al.*, 1994; Mafongoya *et al.*, 1998; Cobo *et al.*, 2002). Significant increase in hibiscus yield in this study was associated to AFPs calliandra mulch. In a prospective study of decomposition rate and the chemical composition of the organic amendments, carried out in same experimental area, calliandra presented a medium- to high-quality litter (1.66% N, 0.16% P, 17.5% lignin; Table 2), and it released nearly 100 kg N and 9 kg  $P ha^{-1} yr^{-1}$  after decomposition (Silva-Galicia *et al.*, 2021). Moreover, the larger proportion of leaves/twigs ratio of calliandra, applied in this study, when compared to the reported by Lehman *et al.* (1995), could promote the nutrient release as leaves usually present more nutrients and low lignin contents than stems

(Lehman *et al.*, 1995; Palm, 1995; Mafongoya *et al.*, 1998). Thus, it is likely that differences in yield between the AFPs may be linked with the amount of nutrients released after calliandra mulch decomposition.

Besides nutrient addition, mulching also has a positive effect on crop performance due to the soil moisture and temperature maintenance (Lal, 1974; Montagnini *et al.*, 1993; Teame *et al.*, 2017). Mulched soils can retain 10–12% more moisture than bare soils (Lal, 1974; Teame *et al.*, 2017), and register more suitable temperatures for crop root. In the present study, soil temperature was not measured, but differences in temperature of  $6.6^{\circ}$ C lower at 5 cm depth between mulched and non-mulched

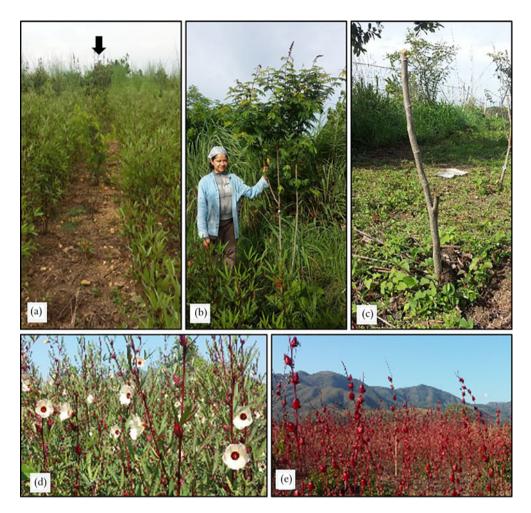


Fig. 3. Calliandra (*Calliandra houstoniana*)-hibiscus (*Hibiscus sadariffa*) alley cropping system. (A) Lines of calliandra (indicated with the black arrow) and alleys of hibiscus at both sides. (B) 18-month-old calliandra tree (2017). (C) Recently cut calliandra tree (at 70 cm height). (D) Hibiscus plantation at flowering. (E) Hibiscus plantation previous cropping.

soils has been reported (Lal, 1974). Mulching can increase infiltration, reduce evaporation and, in general, promote enzymatic activity of soil microorganisms (Roose, 1993; Barrios *et al.*, 2012). Thus, this confirms our initial hypothesis: the combined effect of the physical protection of the mulch of calliandra plus the nutrients it released after its decomposition possibly had an additional positive effect on hibiscus productivity. However, additional experimental work is needed to discern the effect of nutrients, the physical protection or the combined influence on the results obtained.

Nutrient addition after applying high-quality green manure to soil may significantly increase crop productivity, but combining plant material of contrasting quality could modify the nutrient release process (Buckles *et al.*, 1998; Kouyaté *et al.*, 2000; Gartner and Cardon, 2004). In the prospective study developed by Silva-Galicia *et al.* (2021), about the organic amendments employed in *La Montaña*, mucuna released higher amounts of nutrients than that of calliandra mulch (155.3 kg N and 11.6 kg P ha<sup>-1</sup>, Table 2) and, because of that, mucuna was classified as 'high-quality plant material' (Silva-Galicia *et al.*, 2021). Combining plant material of contrasting quality, however, can modify the decomposition process and nutrient mineralization rates compared to species on their own (Montagnini *et al.*, 1993; Handayanto *et al.*, 1997; Gartner and Cardon, 2004).

When plant litters of contrasting quality in an equal proportion are mixed to decompose together, high-quality litters enhance decomposition and mineralization of low-quality plant materials (i.e., synergistic response) (Gartner and Cardon, 2004). On the other side, when the low-quality litter predominates in the mixture, antagonistic responses may be found (i.e., low-quality material decreases the decomposition rate of high-quality species) (Gartner and Cardon, 2004). In the present study, the dry-basis proportion of mucuna (high-quality), calliandra (mediumquality) and hibiscus stover (low-quality) was approximately of 1:4:3, respectively (mucuna: 5.3 t of fresh biomass = 1.3 t of dry biomass). Thus, the relatively poor performance of the AFP3 'hibiscus stover-mucuna' along with the AFP4 'hibiscus stover-calliandra-mucuna' is probably explained because of the non-balanced proportions of the plant material quality applied in these treatments. Further experiments with a higher biomass of mucuna or lower quantity of calliandra and hibiscus stover are needed to test this hypothesis. For example, a mean of 10 t  $ha^{-1}$  of fresh mucuna biomass (approximately 2.5 t  $ha^{-1}$  of dry biomass) was reported to have a significant impact on yield (Kaizzi et al., 2004). In La Montaña, gathering mucuna biomass to initially implement the AFP seems to be feasible, as this species grows vigorously in abandoned plots and on the roadsides (authors' personal observation); and it has been classified as a

high risk of potential invasiveness (CABI, 2022). Collecting mucuna would help reduce its invasion risk, by reducing its population.

It is well known that diazotrophic rhizobacterias and arbuscular mycorrhizal fungi, which were included in the present study as bio-fertilizers can promote growth, vigor and productivity, though this depends on several environmental factors including edaphic and biological conditions (Vessey, 2003; Podile and Kishore, 2006; Cabrera, 2012; Ryan and Graham, 2018; Ahemad and Kibret, 2014). Previous studies on the interaction between hibiscus plants and the arbuscular mycorrhizal fungi R. irregularis showed that hibiscus mycorrhizal associations induced drought stress tolerance by improving the root density and vegetative growth, as well as improved qualitative attributes in calyxes (e.g., anthocyanin content) and also increased yield (Zaliha et al., 2015; Fallahi et al., 2016). The N-fixing bacteria A. brasiliense has also been shown to increase vegetative growth, flower number per plant, quality attributes and yield of hibiscus calyxes (Kahil et al., 2017). Thus, under adequate conditions, biofertilizers should increase hibiscus performance. Apparent null response on yield obtained in this study with bio-fertilizers, could be attributed to the incapacity of these microorganisms to survive and/or effectively colonize the hibiscus plant roots, either by soil conditions or biological factors (Podile and Kishore, 2006; Cabrera, 2012; Ryan and Graham, 2018). For example, after inoculation, the viability of A. brasiliense in soil is determined primarily by the adsorption capacity of clay particles (Bashan, 1999). Bacteria tend to be adsorbed and 'sequestered' by the positively charged clay particles, usually present in acidic soils (as in the study site); thus, the number of available microorganisms for root colonization decreases (Bashan and Levanony, 1988; Bashan, 1999). On the other hand, studies on interactions between mycorrhizal fungi and plant species suggest that parasitic relationships may emerge when exotic fungi and plant species interact (Klironomos, 2003). Thus, edaphic (acidic soil in the study site) or biologic (possible negative interactions between hibiscus landrace and exotic mycorrhizal fungi) may explain the lack of response of the bio-fertilizer treatment on hibiscus yield. Phytochemical studies are needed to verify if this treatment improved other parameters not measured in hibiscus at the end of the trial (e.g., root density or qualitative attributes of the dry calyxes). More importantly, future studies should include measurements of the population density of the microorganisms in question to confirm that they got successfully established in the agroecosystem examined.

Finally, the influence of external factors, such as the rainfall amount or pathogens incidence, may explain the observed differences on hibiscus productivity between years. The total amount of rainfall during 2016 was 1658.5 mm, whereas it was of 1441.2 mm in 2017, this last was considerably lower than the average reported for the study site (1800 mm of annual rainfall, SMN, 2019). It is possible to reject the hypothesis of the effect of competition between hibiscus and calliandra plants on the low productivity of hibiscus in 2017, mainly because, as can be seen in Figure 2, all the plots that had alley cropping of hibiscus with calliandra showed a significantly higher productivity than hibiscus monocultures. Therefore, it can be concluded that the reduction in total rainfall in the period was the main reason for the decrease in hibiscus productivity. In 2018, precipitation surpassed by far the reference value, recording 2309.9 mm of annual rainfall (SMN, 2019); yields obtained, however, were lower than those in the 2016 production. A possible explanation is that, during

mid-September to late-October 2018, due to the excess of precipitation, an important incidence of hibiscus pathogenic fungi was reported for the study site, especially the presence of *Coniella diplodiella* (sin. *Pilidella diplodiella*) and *Corynespora cassicola* (Ortega-Acosta *et al.*, 2015; Noriega-Cantú *et al.*, 2020). Both pathogenic fungi cause a similar symptomatology: circular to irregular sunken blight spots in leaves and calyxes that form necrotic areas when they expand and join, giving a damaged look and calyx desiccation (Ortega-Acosta *et al.*, 2015; Hernández-Morales *et al.*, 2018; Noriega-Cantú *et al.*, 2020). Thus, the fungal infection of hibiscus crop possibly caused a decline in yield during 2018, which, however, remains to be supported by data on disease incidence and severity.

#### Performance of calliandra trees in the alley cropping system

Results found in this study suggest that *C. houstoniana* is a promising option in terms of survival (87.7%), when compared with other species of the same genus (*C. calothyrsus*) displaying more variation in survival rates from 33 to 91% in other alley cropping systems (Evensen *et al.*, 1994; Shepherd *et al.*, 1997; Herbert *et al.*, 2002). Also, it is important to note that calliandra trees did not interfere with hibiscus plant growth nor yield along the trial (2016–2018) (data not shown).

Biomass production is one of the most desired characteristics of a tree in an alley cropping system (Kang et al., 1981). In the present study, the first pruning of C. houstoniana was low  $(0.613 \text{ t ha}^{-1} \text{ in } 2018)$  when compared to those of C. calothyrsus, that ranged from  $1.73 \text{ tha}^{-1} \text{ yr}^{-1}$  (Nolte *et al.*, 2003) to 6.13 t  $ha^{-1} yr^{-1}$  also in the first pruning (Gichuru and Kang, 1989). The low biomass production can be attributed to the relative low tree density in the alleys in this study (2222 trees  $ha^{-1}$ ) when compared with a much denser alley plantation of C. calothyrsus (3947 trees ha<sup>-1</sup>) (Nolte et al., 2003). Thus, additional trials are needed to assess if denser C. houstoniana alleys -as well as more than two prunings per year-increase the biomass production to progressively meet the demand of biomass applied with the AFPs (4.1 t dry calliandra biomass  $ha^{-1}yr^{-1}$ ). Additional to a denser plantation, calliandra biomass may also be gathered from roadsides and secondary forest patches close to the communities, where the species is abundant.

Contrary to other agroforestry systems, in alley cropping it is not possible to obtain significant volumes of timber, because trees are constantly pruned; nonetheless, woody parts can be used as fuelwood or staking material (Kang *et al.*, 1981; Evensen *et al.*, 1994; Arias and Macqueen, 1996; Nolte *et al.*, 2003). This is of great importance, because all people in the region consume fuelwood (Salgado-Terrones *et al.*, 2017), which is often a limited resource (Miramontes *et al.*, 2012).

Summarizing, the most promising fertilization protocol to increase the hibiscus yields was the *AFP4* '*hibiscus-calliandra-mucuna*'. In average, this AFP produced 452.3 kg of dry calyxes ha<sup>-1</sup> during the 3-year trial, more than two times the mean yield acquired in hibiscus crops by the INGO members in *La Montaña* (around 200 kg ha<sup>-1</sup>, *personal communication with growers*) and nearly 48% more than conventional hibiscus cultivation  $(237 \pm 9.5 \text{ kg ha}^{-1} \text{ on average})$ (SAGARPA, 2012). The reason, as discussed above, is that this mixture provides nutrients, organic matter and physical protection compared to simply returning the hibiscus stover—the traditional management—which does not form a complete mulching layer, provides few nutrients and even immobilizes P (Silva-Galicia *et al.*, 2021). If the *AFP4* '*hibiscus-calliandra-mucuna*' continues displaying desirable results, it would be implemented as a formal alley cropping system in farmer's plot in La Montaña; i.e., migrating from the traditional hibiscus-fallow annual cycle to the 'calliandra-hibiscus' alley cropping system managed under agroecological practices. Thus, calliandra trees in contour would provide mulch, while mucuna, the green manure. The hibiscus stover, as it has no other uses among the farmers in La Montaña, may be disposed on the sides of the plots. In fact, some of the members of the INGO cut ditches at the edge of their plots and accumulate all of the stover for a couple of cropping seasons, until that material has decomposed and has been mixed with the retained soil; then they spread this manure onto the soil plot (Galicia-Gallardo et al., 2019). According to a study in Cameroon, farmers willing to adopt the alley cropping system share some features (Adesina et al., 2000). Adopters were male farmers, they belonged to some group and had contact with agroforestry agencies and faced fuelwood scarcity. Similar characteristics prevail in La Montaña: fuelwood is very used and scanty (Miramontes et al., 2012), farmers are members of an INGO-which gives technological and scientific advice-and are familiar with agroecological techniques (Borda-Niño et al., 2017; Hernández-Muciño et al., 2018; Ceccon, 2020); some of them already have trees in their hibiscus plots (Galicia-Gallardo et al., 2019), and nearly 70% of them are men (Galicia-Gallardo, personal communication).

Alley cropping technologies can improve yields and benefit the environment at local and landscape scale, especially in degraded, hilly areas. Studies report that runoff and soil loss in crop plots have decreased and soil fertility has been recovered (Tacio, 1992; Xu *et al.*, 2000). On the other hand, considering the landscape scale, alley cropping systems can play both roles: (i) Space to improve the quality of the agricultural landscape matrix (i.e., as crop production does not rely on agrochemical inputs because of the agroecological practices, land management is considered of low impact) (Fischer and Lindenmayer, 2007; Perfecto and Vandermeer, 2010; Francesconi and Montagnini, 2015; Arroyo-Rodríguez *et al.*, 2020), and (ii) stepping points between forest remnants, because some few woody elements and even scattered trees act as perching-, nesting- or feeding-sites for birds and insects (Manning *et al.*, 2006; Uezu *et al.*, 2008).

# Conclusions

According to the initial hypothesis, amending hibiscus crop plots with a mixture of calliandra mulch and mucuna green manure resulted in highest yield compared to the other AFPs. This find may be related to a combined effect of nutrients supplied and the protective action of the mulch. On the other hand, contrary to the initial assumption, the bio-fertilizer did not have positive effect on yield. Additional experiments are needed to confirm the viability and population density of the microorganisms, both, in the biofertilizer and in the soil after the application.

Calliandra trees can be a promising agroforestry species, especially because of its high survival rate; however, due to the low biomass production, it is recommended to increase the tree density.

Some strategies based on scientific and traditional knowledge, such as those developed in this study, can address part of the socio-ecological problems, either by controlling degradation and/or contributing to the enrichment of biophysical elements that can indirectly improve socio-economic conditions, by increasing crop yield with a low investment of resources.

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