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Contribution of Agroforestry Systems in the Cultivation of Naranjilla (*Solanum quitoense*) Grown in the Amazon Region of Ecuador

Yadira Vargas [1], William Viera [2], Alejandra Díaz [1], Leider Tinoco [1], Julio Macas [1], Carlos Caicedo [1], Marcelo Almeida [3] and Wilson Vásquez-Castillo [4,*]

[1] Central Amazon Research Site (EECA), National Institute of Agricultural Research (INIAP), Joya de Los Sachas 220350, Ecuador
[2] Tumbaco Experimental Farm, Santa Catalina Research Site, National Institute of Agricultural Research (INIAP), Tumbaco 170902, Ecuador
[3] Ciencias Aplicadas, Universidad de Las Américas (UDLA), Redondel del Ciclista, Vía a Nayón, Quito 170124, Ecuador
[4] Ingeniería Agroindustrial, Universidad de Las Américas (UDLA), Redondel del Ciclista, Vía a Nayón, Quito 170124, Ecuador
* Correspondence: wilson.vasquez@udla.edu.ec; Tel.: +593-984-659-247

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Abstract: Agroforestry systems allow conservation of natural resources and promotion of sustainable agriculture in the Ecuadorian Amazon. Nevertheless, the benefit of the associated species that are part of these production systems needs to be demonstrated. The aim of this study was to find out the influence on the crop yield, carbon sequestration, presence of earthworms, and the nutritional contribution of legume species associated with the naranjilla (*Solanum quitoense*) crop in an agroforestry system. The research was carried out in the Palora Experimental Farm of INIAP, using a randomized complete block design with three replications. The treatments were made up of cultivation systems (agroforestry systems with or without 50% fertilization) and monoculture as a control, with two levels of conventional fertilization (50 and 100%). In the agroforestry arrangements, *Gliricidia sepium* and *Flemingia macrophylla* were used to supply biomass. The results showed that during the three evaluation cycles, the yield of naranjilla was influenced by the quality of the biomass added to the soil and not by the amount of synthetic chemical fertilizer that was supplied. The biomass of *G. sepium* and *F. macrophylla* provided a greater amount of Mg, Mn, Zn, B, and Fe; elements that contributed to crop yield and the presence of earthworms. The results suggest that the use of legume species in agroforestry systems positively influenced naranjillla productivity, favoring sustainable agriculture in the Ecuadorian Amazon.

Keywords: biomass; carbon sequestration; earthworms; legumes; nutrients

1. Introduction

The naranjilla fruit (*Solanum quitoense* Lam.) is accepted in the national and international market due to its flavor, aroma, and health compounds namely vitamins (A, B, and C), minerals (calcium and iron), and antioxidants [1]. This fruit has several uses as in the preparation of juices, nectars, carbonated beverages, desserts, sauces, jams, dressings, jellies, and cocktails [2].

This fruit crop is cultivated mainly in the Ecuadorian Amazon [3], with approximately 10,000 hectares destined for production, located in the foothills of the Amazon mountains and plains [4]. The provinces of Napo, Morona Santiago, and Pastaza register the highest production in the Amazon region [3], with yields between 5.49 and 6.04 t ha^{-1} year. The low fruit productivity is due to poor agronomic management. In fact, [1,4] if the crop were to receive adequate and technical management, production would reach up to 30 t ha^{-1} year and a profitability between 119 and 164% would be generated. This profitability is not achieved in the Amazon region, because naranjilla is very reliant on nutrients and in farmers to supply this requirement. Furthermore, the nutrients stored within the surface disappear in the first year of growing, thus in the following plantings, farmers apply more fertilizer to achieve yields similar to the first year [1,5].

Primary forests are cut down to grow this fruit crop. The clearing of these forests and the use of conventional production technologies (high use of chemical input) in the Ecuadorian Amazon cause adverse effects on natural resources, for example a reduction of the biodiversity, degradation, erosion of the soil because of the high application of pesticides, and complete destruction of the ecosystem [1,3,6]. For this reason, new production alternatives that reduce greenhouse gas emissions and enhance crop productivity are being studied [7]. A sustainable alternative is an agroforestry system (AFS), where commercial crops such as fruits and legume trees are combined to improve the economic income of the producers [8].

Carbon storage from the atmosphere is an important issue to reduce the impact of CO_2 emissions which cause serious damage to the environment. There are various strategies for carbon storage, for example plants play an important role since they have the ability to store atmospheric CO_2 [9]. CO_2 storage with enhanced gas recovery can reduce CO_2 emission by sequestrating it into gas reservoirs and simultaneously enhance natural gas production [10]. In this sense, Sun et al. [11] point out that a partial pressure of the gas and the adsorption mechanisms of a binary mixture enable the mechanism of CO_2/CH_4 mixture in a reservoir underground to be understood. In addition, gas hydrates play an important role in the CO_2 capture; thus understanding the pathways of gas hydrate nucleation where the aggregation of molecules is key to the formation of gas hydrates will provide guidance for controlling this strategy [12]. On the other hand, there are several ways to estimate the amount of CO_2 stored but this process is influenced by the type of vegetation, soil, climate, agronomic management, land surface area, and exchange between the vegetation and the soil [13,14].

Carbon is stored more in AFS than monocultures; however, this depends on the environmental, biological, edaphic, and crop management conditions of each site [15–17]. Several studies indicate that an AFS stores twice (34.61 t ha^{-1}) as much carbon (C) as a monoculture (18.74 t ha^{-1}) [18]. In Panama, Costa Rica, and the Peruvian and Colombian Amazon, the quantity of C sequestered in AFS of cocoa with timber and fruit trees (scattered trees) was between 43 and 100 t ha^{-1} per year. In Mexico and Costa Rica, in AFS of *Erythrina poepiggiana* associated with coffee, a storage of C from 115 to 195 t ha^{-1} per year was shown, while it was 93 t ha^{-1} per year in systems with *Gliricidia sepium* and cocoa grown in the Philippines [19,20].

In the Ecuadorian Amazon, there have been few studies on the amount of C stored in AFS. Jadán et al. [21] pointed out that in cocoa AFS, the amount of carbon stored was 141.4 t ha^{-1}. In addition, Vargas et al. [8] determined that in AFS of pitahaya with *E. poeppigiana* and *Flemingia macrophylla* and with *G. sepium* and *F. macrophylla*, it was possible to obtain an acceptable fruit yield (13 and 17 t ha^{-1}, respectively) and high sequestration of C (C:N 15 and 16 ratios, respectively).

In addition to C storage, AFSs also have a potential impact on belowground biodiversity and the physical, chemical, and biological properties of the soil [22]. The population and diversity of earthworms vary according to soil moisture and temperature, soil properties, abundance of litter on the surface, types of vegetation, land use management, human intervention, and microclimate variation [23–26]. In an AFS located in Canada, the number of earthworms was higher near trees (182 individuals m^{-2}) and lower when it was assessed in alleys (between 95 and 117 individuals m^{-2}) [27].

Research on C capture in AFS associated with fruit crops is limited and zero with the naranjilla crop. Considering this situation, the aim of this study was to find out the influence on the crop yield, carbon sequestration, presence of earthworms, and the nutritional contribution of legume species associated with the naranjilla (*Solanum quitoense*) crop in an agroforestry system.

2. Materials and Methods

2.1. Experiment Location

This research was carried out at the Palora Experimental Farm belonging to the Central Research Site of the Amazon of the National Institute of Agricultural Research (INIAP). The experimental plot was in the Palora county in the province of Morona Santiago, at 864 masl of altitude, a latitude of 1°40′14.5″ S, and a longitude of 77°57′50.3″ W. The environmental conditions of this area correspond to a humid subtropical zone. There was an average temperature of 20 °C, an average relative humidity of 89% and 3122 mm year^{-1} of rainfall. This research was carried out in two rainy seasons: a rainy season from February to April with an average precipitation of 900 mm and temperature of 25 °C, and a dry season from August to October with precipitation of 479 mm and temperature of 27 °C.

2.2. Treatments and Experimental Design

The experiment was structured with three replications using a randomized complete block design. The cultivation systems were constituted of agroforestry systems and the control was the monoculture, with two levels of conventional fertilization (50 and 100%). The experimental unit was represented by twenty plants of *S. quitoense*. The total size of the plot was 3500 m^2, with a total of 24 experimental units.

The agroforestry arrangements in alleys were as follows: (1) *F. macrophylla*, (2) *G. sepium*, and (3) *F. macrophylla* + *G. sepium*, all with the *S. quitoense* crop (Figure 1). The species used in this study are nitrogen fixers (N), soil improvers (structure), and macrofauna conservatives [28,29]. Eight treatments were evaluated: three treatments formed by the AFS plus the application of 50% fertilization, another three treatments by the AFS but without fertilization, and two treatments with monocultures and the application of 50 and 100% fertilization. Fertilization percentages are explained in the crop management section. Three cycles of production were evaluated, each comprising 19 months in total but 10 months corresponded to fruit harvesting.

Figure 1. Agroforestry system of *Solanum quitoense* with *Gliricidia sepium*, located in the Palora Experimental Farm.

2.3. Crop Management

The study was implemented in soil where the previous crop was grass. The *S. quitoense* plants were sown at 2.0 m between rows and 2.0 m between plants. *G. sepium* plants were sown at 2.0 m between rows and 1.5 m between plants when alone, and 4 m between rows and 1.5 m between plants in the combined treatment. Meanwhile, *F. macrophylla* was planted at 2 m between rows and 0.5 m between plants when alone, and 4 m between rows and 0.5 m between plants in the combined treatment. A double row was sown for this species.

Basal shoots of *G. sepium* were cut to leave a single stem after six months of planting. The crown formed at four meters from the stem of the plant and the lower branches were pruned in the second year [30]. *F. macrophylla* did not receive any type of pruning. The biomass pruning of *G. sepium* consisted of the elimination of 60% of the biomass from the aerial part of the plant. The quantity of biomass added by this species in the treatments with and without fertilization was from 6 to 7, 13 to 16, and 8 to 9 kg plant^{-1} in the three production cycles. In the *G. sepium* system, biomass incorporation was conducted each 120 days which meant 3 prunings in the year; while in the *F. macrophylla* system, pruning was performed every 90 days (4 prunings a year). *F. macrophylla* plants were pruned when they showed 50% flowering and treatments with and without fertilization from 1 to 2, 1 to 4, and 2 kg plant^{-1} were added in the three production cycles [8]. All the organic matter of the legumes was cut and remained on the surface of the soil alongside the *S. quitoense* plants, following the recommendation of Sánchez-De León et al. [31].

At two months of age from the transplant, training pruning was performed, basal shoots above and below the rootstock were removed, and four secondary branches were chosen to structure the top tree crown. From 2 to 10 months, sanitation and maintenance pruning was carried out with the aim of eliminating branches in the lower area, intertwined branches, overabundance of leaves, branches and fruits showing disease symptoms; this also enabled good air circulation and better entry of light. Pest control was carried out each 15 days in the fructification stage using preventive and curative agrochemicals like cymoxanil, metalaxyl, and abamectin. Weed control was carried out monthly with a brush cutter. The harvest of the fruits for the evaluation of the yield was carried out manually with pruning shears when the fruit was found in the state of maturation 4 according to the Ecuadorian Technical Standard of the Ecuadorian Institute of Standardization [32].

Nutrients were applied according to the recommendation of Revelo et al. [5], taking into consideration the crop requirement to achieve a yield of 30 t ha^{-1}, the soil fertility and its nutrient contribution, as well as fertilizer efficiency.

The fertilization was carried out using nitrate of ammonium containing 33% of N, calcium nitrate with 12.3% of N and 16.8% of Ca, di-ammonium phosphate with 18% of N and 48% of P, sulfate of magnesium with 12% of Mg and 20% of S, and potassium chloride containing 60% of K. In agroforestry systems with 50% fertilization, 86 to 57 g of N, 68 to 75 g of P, 56 to 94 g of K, 38 g of Ca, 19 to 23 g of Mg, and 72 to 90 g of S were added per plant. In the first year, the N was added in highest amount but it decreased in the third year. An amount of 114 to 173 g of N, 135 to 150 g of P, 113 to 188 g of K, 75 g of Ca, 38 to 47 g of Mg, and 144 to 180 g of S were added per plant in the monoculture with 100% fertilization. The same elements were applied to half of the monoculture group with 50% fertilization.

The 20% of N was applied to the transplant and the other four fractions at three, six, eight, and ten months after the transplanting. An amount of 50% of K, P, Mg, Ca, and S was added at the sowing; whereas the other two fractions of K were added at four and eight months later; finally, the other 50% of Mg, Ca, S, and P was added after six months.

2.4. Plant and Soil Analysis

2.4.1. Legume Fresh Biomass

Three plants of *F. macrophylla* and *G. sepium* were taken to quantify the contribution of biomass from pruning. In situ, using scales (model SP2001, Ohaus, Pine Brook, NJ, USA), the total biomass of the pruned parts (leaves and branches) was weighed. The mean of the biomass was multiplied by the number of plants in the plot to estimate the amount of fresh biomass placed in a hectare each year [33]. On the other hand, in the combined treatment, the biomass of the legumes was added to determine the total amount of biomass per hectare per year.

Samples (250 g) combining leaves and branches were obtained during pruning, to carry out chemical analyses to determine dry matter and nutrient concentration (N, P, K, Mg, S, and Ca).

2.4.2. Biomass Nutrient Concentration

To calculate the content of macro- and micronutrients in the legume biomass, the following equation [34] was applied.

$$Q = [MST * X]/10^2 \quad (1)$$

where, Q = Total dry matter nutrient content (kg ha^{-1}), MST = Total dry matter, and X = Dry matter nutrient concentration.

The content of nutrients achieved in the cultivation systems was extrapolated to estimate the contribution of macro- and microelements. Total N was calculated by the semimicro Kjeldahl method [35,36]. The P was estimated by the colorimetric method of the nitric-perchloric digestion extract, while K, Ca, Mg, S, B, Zn, Cu, and Mn were determined by atomic absorption spectrometry [37]. For the determination of total organic C, it was assumed that the C present in the biomass was 50% [38]. The C obtained in each treatment was extrapolated to estimate stocks in units of t h^{-1}.

2.4.3. Soil Nutrient Concentration

Once a year, before implementing the production cycles, soil samples composed of 1 kg were collected for the analyses of organic C, total N, P, K, Ca, Mg, S, B, Zn, and Mn. The determination of N, P, K, Ca, Mg, S, B, Zn, and Mn was carried out following the modified Olsen method, while S and B were obtained from an extract obtained from a monobasic Ca phosphate solution. N, P, and B were determined by colorimetry; K, Ca, Mg, Fe, Zn, Mn, and Cu by atomic absorption spectrometry; and S by turbidimetry [37]. The C:N ratio was determined using the values of C and N.

2.4.4. Number of Earthworms (*Eisenia* sp.)

Soil samples to count the amount of earthworms were taken randomly, they were collected in all treatments (Figure 2). They were collected during their maximum biological activity in April (rainy season) and in October (dry season). In each sampling plot, earthworms were collected from a soil block of 0.5 m × 0.5 m × 0.10 m, corresponding to an area of 0.25 m^2. The sampling was repeated eight times: four samplings were carried out in the rows of naranjilla plants and four in the center of the alley [22,27].

Figure 2. Sampling of earthworms (0.25 m^2) in the naranjilla agroforestry systems, located in the Palora Experimental Farm.

2.4.5. Crop Yield

Nanaranjilla yield was obtained by weighing all harvested fruits and expressed in g plant^{-1}. They were harvested with the degree of maturity 4 (75% yellow-orange color) [32] every 15 days for 300 days.

2.5. Statistical Analysis

R version 4.1.2 software was used for statistical analyses. Variance analysis (ANOVA) was used to identify the influence of treatments and the production cycles on crop yield and on the biomass C:N ratio. The Student–Newman–Keuls method was used with a confidence level of 95% in the post-hoc analyses of the ANOVA test when they were significant; this method was used because it shows greater sensitivity in searching for significant statistical differences.

The stepwise regression algorithms (in their systematic inclusion and exclusion variants) and the Akaike information criterion (AIC) [39,40] were applied in order to select the explanatory variable combinations of crop yield that showed the best relative quality. The forward or systematic inclusion algorithm together with the AIC allowed us to compare linear models by considering each variable as the only regressor. Then the one with the highest relative quality (lowest AIC) was selected, and the process was repeated by including as a second regressor variable, each of those that were not selected in the previous step, and comparing the new models with the previous one. As for the backward or systematic exclusion algorithm, a multiple linear regression model was used, which included all the regressor variables. Each variable was eliminated, and all the AIC values were compared. If it improved in relative quality in relation to the original model, it was used as a new base model and this process continued to be repeated until a model with a single regressor variable was obtained or none of the new resulting models were better than the one selected in the previous step.

Pearson correlation was estimated by relating soil minerals that were found to be significant for crop yield and the number of earthworms within each production cycle. The coefficients were calculated based on the legume species (*F. macrophylla* and *G. sepium*) added to the crop.

3. Results

Significant differences between the production cycles and the AFS were obtained, but no statistical difference was found for the interaction (Table 1).

Table 1. Statistical significance for the individual factors and interaction effect in the fruit yield.

Factor	Yield (t ha^{-1})
Production cycles	*
Agroforestry systems	**
Production cycles × Agroforestry systems	NS

NS: not significant; * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.

The main effect of AFS showed that naranjilla fruit yield decreased in the second cycle of production and slightly increased in the third one (Figure 3).

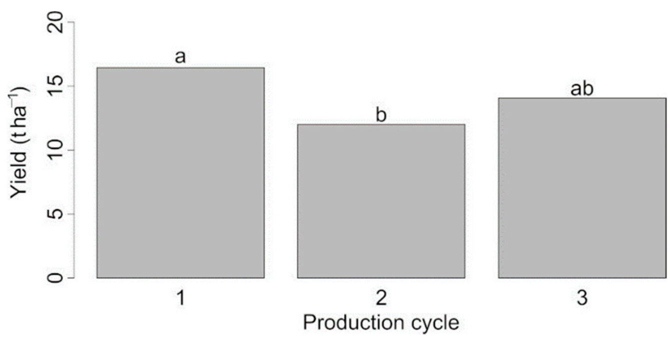

Figure 3. Mean values for fruit yield in the different production cycles. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

AFS showed that the naranjilla crop yield was higher in monocultures with 50 and 100% fertilization and in the AFS that combined the two types of legumes with 50% fertilization (Figure 4). However, it was observed that the treatments that only used a single type of legume with 50% fertilization shared the first range of significance. The lowest yield was obtained in systems using legumes individually but without fertilization.

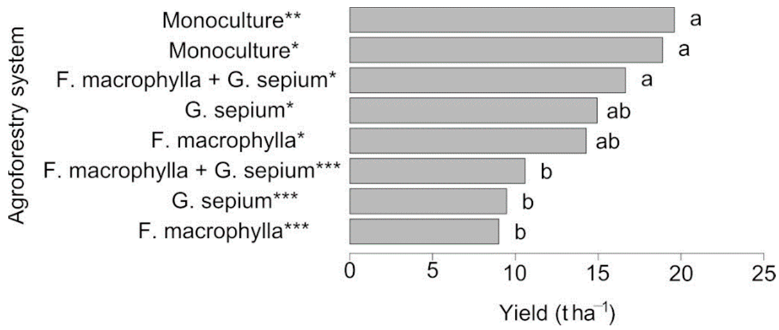

Figure 4. Naranjilla yield achieved by the agroforestry systems. * 50% fertilization; ** 100% fertilization; *** without fertilization. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

The nutrients that most contributed to the yield were Fe, Mg, and B but the last two elements were not statistically significant. This parameter was inversely related to S and the C:N ratio (Table 2).

Table 2. Results of the stepwise regression algorithms to identify the nutrients that contributed to crop yield.

Nutrients	Estimate	Probability
S	−1333.88	2.15^{-5} **
C:N	−192.01	0.001 **
Mg	238.51	0.193 ^{NS}
Fe	5772.76	0.048 *
B	4678.03	0.155 ^{NS}

NS: not significant; * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.

In terms of the C:N ratio, the analysis showed that there were statistically significant differences for the production cycles, the AFS, and their interaction (Table 3).

Table 3. Statistical significance for the individual factors and the interaction effect in the C:N ratio relationship.

Factor	C:N
Production cycles	*
Agroforestry systems	**
Production cycles × Agroforestry systems	*

* significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.

The C:N ratio increased in the treatments that combined the two legumes, while it decreased in the systems that only used one legume species. In addition, a slight increase of this parameter was observed in the second and third cycle of production (Table 4).

The interaction analysis showed that the C:N ratio in the AFS with *G. sepium* + *F. macrophylla* with and without fertilization showed the highest ratio and this value increased in the third cycle. The AFS using just one legume species showed variable behavior. For *F. macrophylla*, it was observed that the ratio increased in the second cycle and slightly decreased in the third cycle; whereas for *G. sepium*, the ratio slightly decreased in the second cycle and increased in the third cycle (Table 5).

Table 4. C:N ratio obtained for the production cycles and agroforestry systems.

Agroforestry System	Production Cycle	C:N
F. macrophylla + *G. sepium* *		28.90 a
F. macrophylla + *G. sepium* ***		28.53 a
F. macrophylla ***		16.15 b
F. macrophylla *		15.83 b
G. sepium ***		12.73 c
G. sepium *		12.71 c
	1	13.44 b
	2	14.52 a
	3	15.11 a

* 50% fertilization; *** without fertilization. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

Table 5. C:N ratio obtained for the interaction between the production cycles and the agroforestry systems.

Agroforestry System	Production Cycle	C:N
F. macrophylla + *G. sepium* ***	1	26.44 b
F. macrophylla + *G. sepium* *		26.81 b
F. macrophylla ***		15.60 de
F. macrophylla *		14.16 defgh
G. sepium ***		12.51 fgh
G. sepium *		12.04 gh
F. macrophylla + *G. sepium* ***	2	28.67 ab
F. macrophylla + *G. sepium* *		28.95 ab
F. macrophylla ***		18.28 c
F. macrophylla *		16.99 cd
G. sepium ***		11.93 gh
G. sepium *		11.38 h
F. macrophylla + *G. sepium* ***	3	30.48 a
F. macrophylla + *G. sepium* *		30.97 a
F. macrophylla ***		15.89 de
F. macrophylla *		15.07 def
G. sepium ***		14.71 defg
G. sepium *		13.77 efgh

* 50% fertilization; *** without fertilization. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

Regarding the amount of stored C, the analysis showed that there were significant differences for the production cycles and their interaction (Table 6).

Table 6. Statistical significance for the main effects and interaction for the stored C.

Factor	C
Production cycles	*
Agroforestry systems	NS
Production cycles × Agroforestry systems	*

NS: not significant; * significant at $p \leq 0.05$.

The amount of stored C varied from 0.60 to 3.43 t ha^{-1} in the three production cycles. It was observed that the greatest amount of stored C was achieved in the second production cycle in the AFS with *G. sepium* (Table 7).

Table 7. Mean values for the stored C in the different production cycles and agroforestry systems.

Agroforestry System	Production Cycle	Stored C
F. macrophylla + G. sepium ***	1	0.63 g
F. macrophylla + G. sepium *		0.60 g
F. macrophylla ***		2.10 bcde
F. macrophylla *		1.93 cdefg
G. sepium ***		0.90 efg
G. sepium *		0.73 fg
F. macrophylla + G. sepium ***	2	3.00 abc
F. macrophylla + G. sepium *		3.23 ab
F. macrophylla ***		1.70 defg
F. macrophylla *		1.67 defg
G. sepium *		2.53 abcd
G. sepium ***		3.43 a
F. macrophylla + G. sepium ***	3	0.80 fg
F. macrophylla + G. sepium *		1.13 efg
F. macrophylla ***		2.50 abcd
F. macrophylla *		2.90 abcd
G. sepium ***		1.83 cdefg
G. sepium *		2.07 bcde
	1	1.15 c
	2	2.59 a
	3	1.87 b

* 50% fertilization; *** without fertilization. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

The analysis showed that there were highly significant statistical differences for the number of earthworms per production cycle in the two sampling seasons (rainy and dry season). Significant differences were also found between the AFS and the seasons. No interaction was found between sampling season and AFS. The number of earthworms was higher in the first cycle; it decreased by 34.4 and 29% (rainy and dry season, respectively) in the second cycle; and finally, it increased in the third cycle in both evaluation seasons (30 and 70%, respectively) (Figure 5).

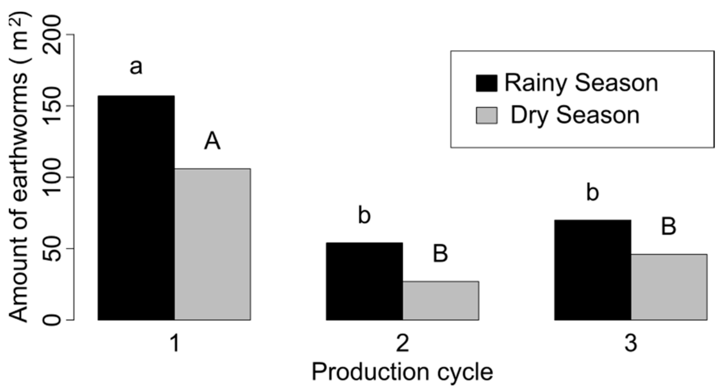

Figure 5. Mean values of the number of earthworms determined in the agroforestry systems in the different production cycles. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

The highest abundance of earthworms was found in the AFS with *F. macrophylla* without fertilization in the two assessed seasons, while the lowest amount was found in the monoculture with 50% of fertilization (Table 8).

Table 8. Amount of earthworms determined in the agroforestry systems in the two seasons.

Agroforestry System	Season	Amount of Earthworms (m^2)
F. macrophylla ***	Rainy season	143.89 a
G. sepium ***		109.33 ab
G. sepium *		106.78 bc
F. macrophylla + G. sepium ***		98.44 bcd
F. macrophylla + G. sepium *		85,00 bcde
F. macrophylla *		73.67 cde
Monoculture ***		70.44 de
Monoculture *		60.00 e
F. macrophylla ***	Dry season	91.67 a
G. sepium ***		70.67 ab
G. sepium *		64.22 ab
F. macrophylla + G. sepium ***		62.44 ab
F. macrophylla + G. sepium *		54.89 bc
F. macrophylla *		54.78 bc
Monoculture **		47.33 bc
Monoculture *		29.78 c

* 50% fertilization; ** 100% fertilization; *** without fertilization. Different letters indicate significant differences ($p < 0.05$) using the ANOVA one-way analysis followed by Tukey's test.

The soil nutrients that positively influenced the number of earthworms were Mn and B in the rainy season, but the last element was not statistically significant, while in the dry season they were B and Zn (Table 9). On the other hand, elements such as P, Cu, Ca, and N had a negative influence on the number of earthworms in the two evaluation seasons.

Table 9. Results of the stepwise regression algorithms to identify the nutrients that contributed to the amount of earthworms.

Season	Nutrient	Estimate	Probability
Rainy season	Mn	2.11	1.75^{-5} **
	P	−2.61	0.07 NS
	B	89.44	0.17 NS
Dry season	Cu	−2.61	0.001 **
	B	164.12	0.019 *
	Ca	−8.58	0.005 **
	Zn	3.65	0.034 *
	N	−48.53	0.131 NS

NS: not significant; * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.

The regression analysis considering the biomass, soil nutrients, and amount of earthworms is shown in Table 10. In terms of F. macrophylla, the amount of biomass added in the first cycle was between 1 and 2 kg plant^{-1}, showing a relatively high (−0.64) and high (−0.81) inverse relationships among the Zn and Mg with the number of earthworms, respectively. In the second cycle (1 to 4 kg kg plant^{-1}) and third cycle (2 kg plant^{-1}), B showed a slight correlation (0.59 and 0.58 respectively). Turning to *G. sepium*, where 6 to 7 kg plant^{-1} was added in the first cycle, a relatively high correlation (0.79) was observed between the Zn with the number of earthworms. A high inverse correlation (−0.83) was shown in the second cycle (13 to 16 kg plant^{-1}) between the Mg with the number of earthworms; while in the third cycle (8 to 9 kg plant^{-1}), B showed a strong correlation (0.92) between B with the number of earthworms.

Table 10. Correlation coefficients to determine the relationship among the biomass (legume), soil nutrient, and amount of earthworms.

Legume Species	Nutrient	Cycle 1	Cycle 2	Cycle 3
F. macrophylla	Earthworms vs. Mg	−0.81	0.06	−0.18
	Earthworms vs. Zn	−0.64	−0.09	0.21
	Earthworms vs. B	0.29	0.59	0.58
G. sepium	Earthworms vs. Mg	0.31	−0.83	0.34
	Earthworms vs. Zn	0.79	0.19	−0.36
	Earthworms vs. B	0.49	−0.05	0.92

4. Discussion

In this study, the naranjilla yield was 16.44 t ha^{-1} in the first production cycle, which decreased to 12.01 in the third production cycle. The variation in yield in the different production cycles was possibly due to the fact that naranjilla is a very demanding crop for nutrients, as pointed out by Viera et al. [1,5] who affirm that the yield is greater in the first year of the crop establishment because the plant takes advantage of all the available nutrients in the soil. This same behavior was found when this crop was associated with duraznillo (*Prunus annularis*) due to competition for soil nutrients [41].

In addition, it was determined that the AFS that combines the two types of legumes (*F. macrophylla* + *G. sepium*) with 50% fertilization showed a yield (16.65 t ha^{-1}) that was statistically similar to the yield obtained by the monocultures with 50 and 100% fertilization (18.89 and 19.60 t ha^{-1}, respectively). This would indicate that this fruit crop grown under an AFS is able to maintain production relatively similar to monoculture (even 100% fertilization) but with the environmental benefits that this type of cultivation system provides.

In this study, the yields obtained by the monocultures (50% and 100% of fertilization) exceeded the national average yield (5.49 to 6.04 t ha^{-1} year) reported for naranjilla cultivated in monoculture [3,4]. This behavior is due to the fact that the naranjilla received adequate agronomic management (pruning, fertilization, and phytosanitary controls). This is corroborated by Torres-Navarrete et al. [4], who mentioned that when the naranjilla receives efficient and timely management, a profitability of 119% is generated. In addition, it was determined that the yields obtained in AFS with 50% fertilization and without fertilization were in the same range of significance as monocultures and also exceeded the yield reported for the national average. This same favorable behavior of yield increase has been found in other AFSs, for example in a system of *Hylocereus megalanthus* with *E. poeppigiana* and *G. sepium*, where the yields exceeded 33 and 50% (respectively) in comparison to the monoculture [8]. The same occurred in a plantation of *G. sepium* associated with corn (*Z. mays*) and sorghum (*Sorghum* sp.), where the yields exceeded 42 and 55% (respectively) in comparison to the yield reached by the monoculture [42]. Kebede [43] also found that *Z. mays* cultivated in legume fallow increased the yield by fourfold in the fourth year. In addition, it was reported that the yield improved by 20% in an AFS of *G. sepium* + *Leucaena leucocephala* + *E. variegate* associated with coffee [44]. Therefore, these results indicate that incorporating legume species alongside the crop causes the yield to increase.

On the other hand, Borja et al. [45] point out that when naranjilla was associated with more than three fruit species (*Musa cavendishii*, *Theobroma cacao*, and *Borojoa patinoi*), fruit production decreased. Therefore, based on the results of this study, it is better to associate the naranjilla crop with legume species that provide biomass to increase the nutrient content of the soil.

In this study, the nutrients coming from the legume biomass that contributed the most to the naranjilla yield were Fe, Mg, and B. Fe deficiency can limit crop yield and Mg is an element related to fruit quality in the naranjilla crop [46]. The legume biomass incorporated into the soil decomposes and provokes the solubilization of macronutrients and micronutrients, alleviating nutrient deficiency through recycling processes [43,47]. Viera et al. [1] mentioned that the yield improves when Mg is part of the complete fertilization (all nutrients) of the naranjilla crop, but this element does not limit the naranjilla yield. In addition, Vargas-Tierras et al. [8] found that in an AFS of *H. megalanthus* with *E. poeppigiana* and *G. sepium*, the fruit yield is directly related to the availability of Ca and Mg from the legume biomass.

According to the results, S and the C:N ratio did not influence the crop yield of this solanaceous crop. This behavior has been corroborated by Viera et al. [1] and Karagatzides et al. [48], who reported that S does not limit the production of *S. quitoense* and *S. tuberosum*. The C:N ratio is mainly related to the decomposition and release of nutrients by legumes; therefore, the C:N ratio in all agroforestry systems was within the acceptable range (<30) for net N mineralization to occur and adequate residue decomposition [49,50].

In this study, it was also found that the C:N ratio was higher (>28) in the treatments that combined the two legumes. This value is possibly due to the fact that the microorganisms consume the stubble quickly and would not need much additional N to decompose the residues. Vigil & Kissel and Mao et al. [49,51] reported that if the C:N ratio is high, there are high decomposition rates and low availability of N for the decomposing microorganisms, and that the formation of the N-lignin complex is stimulated, accelerating the decomposition rates.

In the AFS where a single type of legume was used, the C:N ratio was lower (12 to 16); this effect was also reported by Vargas-Tierras et al. and Camelo et al. [8,52]. They mention that when the C:N ratio is less than 20, mineralization is favored before nutrient immobilization. In addition, the microorganisms consume the legume and leave the excess of N available for the crop or for use in the decomposition of other types of residues.

It was observed that the C:N ratio increased in the production cycles; different behavior was reported by Vargas-Tierras et al. [8] in a AFS with *H. megalanthus* where the C:N ratio slightly decreased from 15.80 (year 1) to 14.53 (year 4).

The interaction of the AFS and the production cycles showed that the AFS with *G. sepium* + *F. macrophylla* showed an increase in the C:N ratio; the C:N ratio was variable in the AFS with *F. macrophylla*; while in the AFS with *G. sepium*, the C:N ratio decreased in second cycle 2 and increased in the third cycle. These variations could possibly be due to the fact that the decomposition process is not only based on the C:N ratio, but also depends on the distribution of plant material on the soil surface, humidity, and the decomposing microorganisms present on the ground [53,54].

The amount of stored C by the aerial biomass of legumes was higher in the second production cycle compared to the other cycles in all AFS. Similar amounts of stored C were found in AFS of coffee with *E. poeppigiana* and *Inga densiflora*, with the aerial biomass of the legumes stored from 1.7 to 3.1 and from 2 to 4.6 t ha^{-1} of C, respectively [55,56].

The amount of earthworms varied in the different cycles of production; this behavior might be related to the amount of organic matter contained in the soil. Price & Gordon [57] reported that when the amount of organic matter is high, the number of earthworms increases. In addition, Blakemore [58] found that the presence of earthworms is high when quality food (manure and legumes) is available. A fact to consider in this study is that the amount of the biomass added of *G. sepium* was higher than *F. macrophylla*, thus this would influence the quantity of organic matter as well as the amount of incorporated nutrients.

The positive difference in the abundance of earthworms in the AFS in relation to the monoculture could be explained by the microclimate variations created around the legume species. Another factor that may have contributed to the distribution of earthworms in the AFS could be the legume's high N content, making them a highly palatable resource for earthworms [59]. Price & Gordon [57] reported that the content of nutrients in the soil and microclimate formed by the tree species cause the density and biomass of the earthworms to increase. The opposite happens in monocultures, where a depletion of the earthworm population is observed [58], which occurred in this study in the monocultures, where lower amounts of earthworms were recorded. On the other hand, the lower number of earthworms found in the dry season is possibly due to the fact that when temperatures were higher and rainfall was reduced, earthworms migrate to the deeper layers of soil and form drought-resistant cocoons [57].

The presence of nutrients such as Mn, B, and Zn in the AFS soil could be due to the fact that earthworms remove these nutrients from the deeper layers and deposit them in the upper layer [60]. The positive influence of Zn on the abundance of earthworms might be because they are epigeal (develop on the ground) and prosper in organic residues that have Zn concentrations of between 79 and 607 mg kg^{-1} [61,62].

Ahmed & Al-Mutairi [60] pointed out that inorganic fertilizers can have a positive effect on the population of earthworms but are also detrimental to them because they increase the number of earthworms by increasing vegetative production but also reduce their number by lowering the pH. The addition of biomass to the soil favored the presence of earthworms and the release of important macro- and micronutrients for the cultivation of the naranjilla crop. In this study, the application of N, P, and Ca through the use of synthetic chemical fertilizers caused negative effects on earthworms, possibly due to the fact that the soil became a little more acidic (pH around 5.3). Lalthanzara [63] found that the long-term application of inorganic fertilizers can negatively affect earthworm populations due to soil acidification or other changes in the soil. Blakemore [58] mentions that earthworms were completely eliminated in plots with a pH of less than 4 and fertilized with N, P, and K.

The negative effect of Cu on the earthworm population might be because during the naranjilla crop cycle, several applications of Cu were made to prevent diseases; this activity possibly caused the accumulation of this element in the soil from the litter of the crop that was deposited on the ground and decomposed over time. This behavior was also found by Blakemore and Ahmed & Al-Mutairi [58,60], who mentioned that when leaves or plants contaminated with Cu are dumped on the ground, they can become a danger to earthworms.

5. Conclusions

AFS with naranjilla in the Ecuadorian Amazon constitute a sustainable alternative for its production because the yield obtained with and without fertilization exceeded the national average production by more than 50%. In terms of yield, several of the evaluated AFSs were not statistically different to the monoculture, which means that both systems are productive, but the AFS can provide intangible environmental benefits, such as minimizing the expansion of the agricultural frontier and the reduction of the use of agrochemicals.

The patterns of the C:N ratio found in the AFS showed that the legume species used in this research increased this parameter, and thus the C sequestration improved, which is environmentally beneficial.

In the AFS, the quantity of added biomass influenced the amount of nutrients incorporated into the naranjilla crop. Results also showed that the legume biomass had a relationship with the soil nutrients and the number of earthworms. Therefore, these events allow the suggestion that the legume species used in this study might be considered as a good source of nutrients for this fruit crop because they incorporated Mg, B, and Fe, which contributed positively to crop yield; and they were also related to soil nutrients such as Zn that benefit the presence of earthworms.

The decrease in earthworms in monocultures shows the need to evaluate conventional methods of agricultural production in the Ecuadorian Amazon, with the aim of promoting studies that enable the restoration of soil fauna (mainly earthworms) that allow soils to be rebuilt and contribute positively to the production systems. The use of sustainable production systems should be promoted through the use of AFS in the Ecuadorian Amazon in order to restore biotic abundance and reduce the loss of biodiversity.

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