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Nutrient Contribution and Carbon Sequestration of an Agroforestry System of *Coffea canephora* Cultivated by Conventional and Organic Management in the Ecuadorian Amazon

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Abstract: Agroforestry systems (AFSs) seek synergies that improve productivity, sustainability, and environmental benefits. This is achieved through the supplying of nutrients to the soil, carbon storage, and sequestration. In the Ecuadorian Amazon, *Coffea canephora* is planted together with leguminous, woody, forest, and secondary forest species, where the continuous incorporation of vegetative residues from shade species represents a substantial addition of nutrients within these systems. This study was carried out from 2018 to 2022 to determine the contribution of nutrients contained in the biomass and C sequestration in agroforestry systems of coffee with conventional (high use of agrochemicals) and organic (without the use of chemicals) management. The study was carried out with a randomized complete block design, using a factorial arrangement (2 × 4 with three replications). This arrangement included two types of systems (agroforestry and monoculture) and four agronomic management practices (high and medium for conventional, and intensive and low organic). The biomass and nutrient content were measured twice a year (every 180 days); in addition, the yield was also recorded. A multivariate and univariate analysis was used for data analysis through R and SAS software. After five years of evaluation, it was determined that the N, K, Ca, and Mg contents were higher in the agroforestry systems than the monocultures. In the AFSs, the highest nutrient content was obtained with the medium conventional and low organic agronomic management, while in the monocultures, it was obtained with the high and medium conventional management. In addition, at a soil depth of 20 cm, the total storage and CO_2 were 38.12 and 139.8 t ha^{-1}, respectively. The highest yields were obtained with conventional management in AFSs (1599 kg ha^{-1}) and monoculture (1789.45 kg ha^{-1}). Overall, AFSs showed a significant contribution of nutrients, such as N, K, Ca, and Mg, for coffee cultivation; moreover, yields were similar in the AFS and monoculture with both conventional and organic management, which is positive, since AFSs also contribute environmental benefits.

Keywords: biomass; nutrients; C storage; C sequestration

1. Introduction

Climate change and land degradation in tropical regions constitute a latent problem [1] due to their significant impact on agriculture, biodiversity, climate patterns, and water security [2]. The conversion of native forests to cropland leads to a rapid decline in soil nutrient cycling [3]. In response, agricultural workers often apply significant quantities of mineral fertilizers to counteract the low fertility of tropical soils that have a high nutrient-binding capacity [4]. This behavior has prompted several countries to focus their research on finding solutions to help restore soil fertility and restore soil organic carbon reversals [1].

Agroforestry systems (AFSs) can mitigate climate change and encourage nutrient cycling. Agroforestry encompasses traditional and modern systems that integrate trees with crops and/or animals [2,5], help sustain production over time, mitigate greenhouse gas effects, and address food security [5]. In addition, these systems are productive and absorb substantial amounts of CO_2 from the Earth's atmosphere, and have been supported by several studies, highlighting their potential for carbon sequestration and storage in standing vegetation, soil organic matter, and harvested biomass products [6].

Agroforestry often yields contradictory results, e.g., some studies report an increase in soil organic carbon in silvopastoral systems [7,8] in multi-strata home gardens, while other studies say the opposite [9]. In a Brazilian biome, it was determined that ecosystem services and nutrient cycling in biodiverse AFSs were higher than they were in monocultures; however, soil organic carbon regulation and storage services were lower in simple AFSs [10,11]. Although several hypotheses have been put forward, such as soil texture influencing organic carbon storage in tropical AFSs, conclusive evidence is still lacking [11]. Given this scenario, there are still gaps in our knowledge concerning organic carbon storage and nutrient cycling in AFSs [1].

On the other hand, forest species in AFSs incorporate biomass into the soil, causing organic matter levels to increase, as well as the microbial activity; in addition, forest species fix atmospheric nitrogen (N), and others protect the soil from erosion caused by water and wind, minimizing nutrient loss and improving soil fertility [12–14]. In addition, the incorporation of important nutrients for the growth and development of the main crop takes place [15]. Montenegro [16] notes that multipurpose species help to extract nutrients from deeper soil layers and provide shade for crops. Filho et al. [17], following 10 years of research, determined that the coffee agroforestry system with *Erythrina poeppigiana* was efficient because it produced 10,000 kg ha^{-1} yr^{-1} of biomass, which contributed to enhancing the physical and chemical properties of the soil, avoided acidification, and produced higher earthworm abundance and biomass than in monocultures. Fernández-Ojeada et al. [18] found that the quantities of organic matter, N, and phosphorus (P) in the soil surface (0–10 cm) increased by 11.92, 0.59, and 16.9%, respectively. In addition, the contents of organic matter (5.34%) and total N (0.26%) were higher in AFSs than in monocultures (4.63 and 0.23%, respectively) [18].

Likewise, in the Ecuadorian Amazon, some studies show that, in AFSs of *Theobroma cacao*, *Solanum quitoense*, and *Selenicereus megalanthus*, the presence of biomass positively affected the availability of N, potassium (K), magnesium (Mg), and calcium (Ca) [2,5,19]. Chavez et al. [20] mention that, in coffee (*Coffea canephora*) plantations, legumes provide more than 350 kg ha^{-1} of N and improve the availability of P and K in the soil. Ramírez and Küsters [21] consider that K and Ca in coffee cultivation are considered nutrients that contribute toward improving the yield, water use efficiency, and adaptation to climate change. In addition, Schmidt et al. and Byrareddy et al. [22,23] point out that N, K, Ca, and Mg are essential nutrients that affect the vegetative and reproductive growth cycle of coffee, as well as its productivity.

On the other hand, several studies have shown that AFSs store more C than open-field crops and pastures [24]. For example, coffee associated with *Inga* spp., *Eucalyptus* spp., and *Pinus* spp. produces more carbon dioxide (189, 120–146, 162–178 t C ha^{-1}, respectively) than in a monoculture (100–113 t C ha^{-1}) [25]. In coffee plantations associated with *E. poeppigiana*, the amount of carbon stored was 115 and 195 t ha^{-1} yr^{-1} [26,27]. In the Ecuadorian Amazon, in AFSs of *T. cacao* and *S. quitoense*, the total carbon stored was between 33 and 42 and 0.60 and 3.43 t ha^{-1}, respectively [2,19].

The interest in growing coffee in the Ecuadorian Amazon is due to the fact that more than 60% of small- and medium-sized non-Indigenous and Indigenous (Kichwa and Shuar) people grow coffee under AFSs [28,29]. Furthermore, Ecuador is considered a producer of quality coffee, owing to its geographical position and environmental conditions (climate and altitude). [30]. In addition, in 2020, 1% (69,788.47 USD) of non-oil exports corresponded to exports of coffee and its processing as a primary (9.82%) and industrialized (90.2%) product [31]; it is estimated that global coffee consumption will increase by 1.9% in the next few years [31]. Also, in the last 5 years, in the Ecuadorian Amazon, a contest called "The Golden Cup" has taken place, involving the Robusta variety of coffee grown under AFSs, an event that has made it possible to identify quality coffee (excellent aroma and good flavor) in the province of Orellana. This coffee is being marketed in the USA and numerous European nations [32].

Finally, the generation of sustainable production technologies in the Ecuadorian Amazon has made it possible to identify AFSs with productive and agroecological potential, because the soil in the region is suitable for forestry activities and not for conventional agricultural activities [2,5]. Hence, this study's objective was to determine the nutrient content in biomass and carbon sequestration in AFSs and monocultures of *C. canephora* subvar. robusta (L. Linden) A. Chev.

2. Materials and Methods

2.1. Experiment Location

The research was carried out at the Central Amazon Research Station (EECA) of the National Institute of Agricultural Research (INIAP) in La Joya de los Sachas canton in the province of Orellana. This experimental station is situated at 00°21′31.2″ S latitude and 76°52′40.1″ W longitude (Figure 1) at an altitude of 250 masl. Given its location in a tropical rainforest (bhT) [33], it has a humid subtropical climate, an average rainfall of 3050 mm yr^{-1}, an average annual temperature of 25.5 °C, and a relative humidity of 84 to 95% [29].

2.2. Experimental Treatments

The trial was arranged under a randomized complete block design with three replications and a factorial arrangement (2 × 4), which consisted of the arrangements (agroforestry system and monoculture) and four agronomic managements (two conventional and two organic). The experimental unit was represented by 144 coffee plants with 9 multipurpose trees (various agricultural uses: nitrogen fixation, biomass contribution, improvement of soil conditions, etc.). The total plot size was 1080 m^2, with a total of 24 experimental units. Evaluations were conducted for 5 consecutive years, from 2018 to 2022. For the establishment of the agroforestry arrangements, nitrogen-fixing trees were used as follows: *E. poeppigiana* + *Myroxylon balsamum* with coffee cultivation and coffee in full sunlight (monoculture) (Table 1). The forest species used had an oval crown shape [34] and were rounded [35], respectively.

The agronomic management levels corresponded to medium and conventional high management using pesticides of toxicological categories II, III, and IV, synthetic chemical fertilizers, and chemical and mechanized weed control. The difference was that, in the conventional high management, 50% more fertilizer was used, and the weed control was chemical (every 2 months, 6 applications per year), whereas, in the conventional medium management, the weed control was chemical (2 applications per year, every 6 months) and mechanized (every 1.5 months, 6 applications per year). The other management methods used were low organic and intensive organic (fertilizer from organic sources, copper-based pesticides, and mechanized weed control). Fertilizer use was reduced by 50% in the low organic management when compared to the intensive organic management, and the weed control was carried out every three months, while, in the intensive organic management, it was monthly.

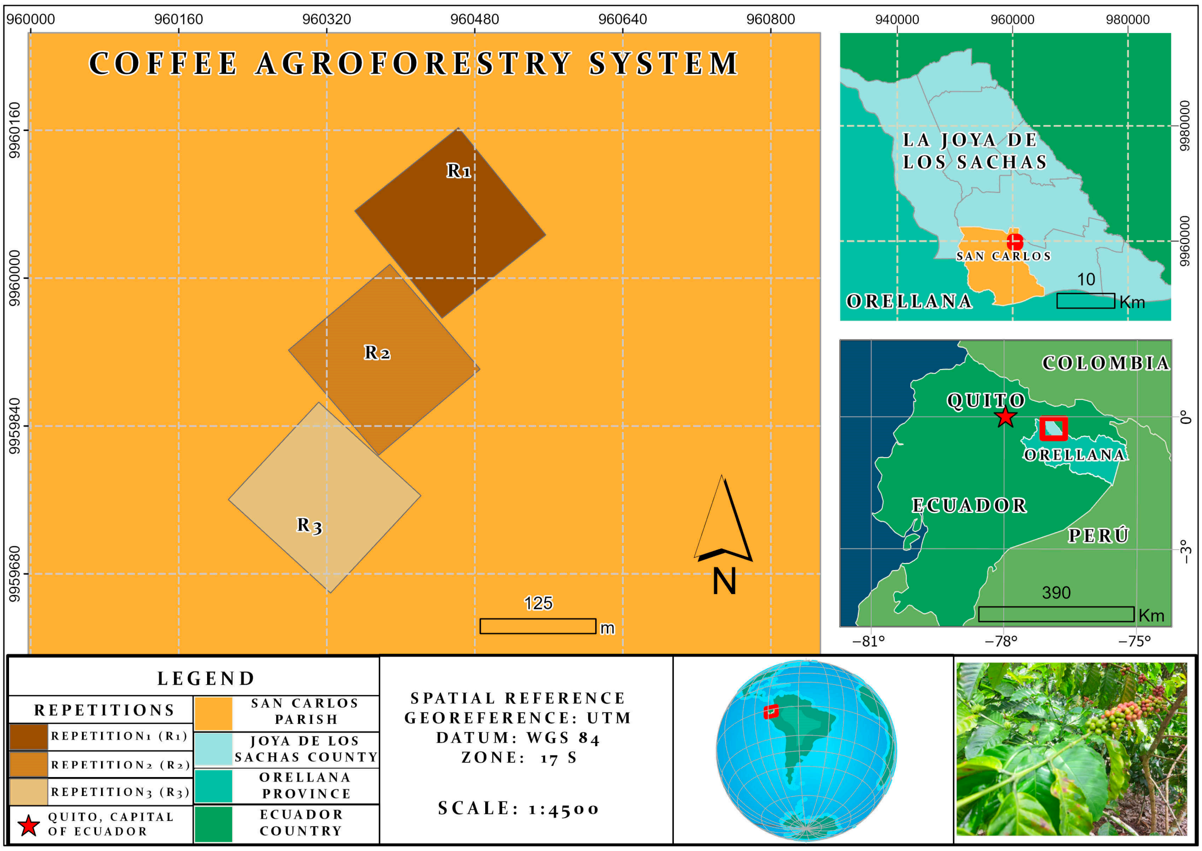

Figure 1. The geographical localization and distribution of the coffee agroforestry system trial in La Joya de los Sachas, Orellana, Ecuador.

Table 1. Treatments evaluated for the coffee agrosystems.

Treatment	Arrangement	Agronomic Management
T1	*E. poeppigiana* + *Myroxylon balsamum*	Conventional high
T2		Conventional medium
T3		Intensive organic
T4		Low organic
T5	Monoculture	Conventional high
T6		Conventional medium
T7		Intensive organic
T8		Low organic

2.3. Crop Management

The research was conducted on a coffee agroforestry system comprised of three-year-old *Coffea canephora* plants. Coffee bushes were planted with a spacing of 3 m between rows and 2.5 m between individual plants. Additionally, multipurpose *M. balsamum* and *E. poeppigiana* trees were transplanted with a spacing of 6 m between rows and 10 m between trees.

In 2018, the multipurpose trees underwent pruning, trimming away lower branches and shaping the canopy from a height of 4 m between plants [36]. In the same year, maintenance pruning was carried out, eliminating additional stems from the coffee plants, and every three months, regular maintenance pruning (elimination of new stems) was carried out [37]. After the harvest, vegetative shoots and broken and diseased branches were removed without affecting the yield or the physiology of the coffee plants [38].

The biomass pruning of the shade species entailed removing 50% of the aboveground biomass. The amount of biomass incorporated ranged from 12 to 21 kg plant^{-1} for *M. balsamum* and 7 to 18 kg plant^{-1} for *E. poeppigiana* between the third and fifth year of incorporation. Biomass incorporation was performed every 180 days (2 pruning sessions per year) [15,16]. This frequency of pruning prevents the legume from competing with the crop (by solar radiation) and the plant recovers quickly, increasing the amount of biomass. All of the organic matter was cut up and left upon the surface of the soil beside the coffee plants, following the recommendations of Vargas et al. [5].

In the first year, four training pruning sessions were carried out in order to select two to three orthotropic axes in the coffee crop. From the second year onwards, the elimination of unproductive stems was carried out, which allowed for good air circulation, thus reducing the propagation of pathogens [29]. The quantity of nutrients applied was established based on crop needs, soil nutrient supply based on soil fertility, and fertilizer efficiency [39].

In conventional management, the fertilizers used were ammonium nitrate (34% N), potassium nitrate (13% N, 46% K), mono potassium phosphate (52% P, 34% K), magnesium nitrate (10% N, 15% Mg), YaraMila Actyva (20% N, 7% P, 10% K), and YaraMila Complex (12.4% N, 11% P, 18% K, 2.7% Mg). Altogether, 38 to 67 g plant^{-1} of N, 47 to 25 g plant^{-1} of P, and 60 to 67 g plant^{-1} of K were administered in the conventional medium management, and 50% more N, P, K, and Mg in the conventional high management. In organic management, organic fertilizer was used (1.63% N, 2.9% P, 3.19% K, 4.18% Ca, 1.6% Mg). In the low organic management (BO), 500 g plant^{-1} of organic fertilizer was applied, and, in the OI, the dose was doubled. Crop fertilization was carried out only once a year.

Starting in 2018, 1 kg composite soil samples were taken in identified plastic bags and transferred to the EECA Soil and Water Laboratory to perform the analyses of organic C, macronutrients, and micronutrients based on the methodology described in Official Methods of Analysis (AOAC) [40].

The assessment of biomass contribution from pruning involved weighing (in kilograms) the total pruning biomass (including leaves and branches) on site for each treatment using a scale (model SP 2001, Ohaus, Ciudad de México, Mexico). To work out the fresh biomass per hectare, the average biomass per tree was calculated and multiplied by the number of trees per hectare [16]. Composite 250 g samples (including leaves and branches) were collected during each pruning session, placed in labeled paper bags, and sent to the EECA Soil and Water laboratory for nutrient analysis.

2.4. Study Variables

2.4.1. Nutrient Concentration in Biomass

The calculation of the N, K, Ca, and Mg content in % was determined via multiplying the total biomass produced per treatment with the dry matter that was produced by each legume species, then applying the equation suggested by Jiménez et al. [41] for macroelements.

$$Q = \frac{[MST \times X]}{10^2}$$

Q = Nutrient content in total dry matter (expressed in kg nutrient ha^{-1});
MST = Total dry matter;
X = Nutrient concentration in dry matter.

The nutrient content obtained from each of the treatments was extrapolated to estimate the annual supply of nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg) in kilograms per hectare. The total N was determined using the Semimicro Kjeldahl method [42] while K, Ca, and Mg were determined via atomic absorption spectrometry [5]. To determine the total organic carbon, 50% of the carbon present in the biomass was considered [43].

2.4.2. Estimation of Carbon Content

To estimate the carbon that was obtained with each treatment, stocks were extrapolated to tons per hectare. The following formula was employed to determine the soil C content at a depth of 0–20 m:

$$COS = \%\ SOF \times Da \times P \times 100$$

where *COS* = soil organic carbon (t ha^{-1}); % *SOF* = soil carbon fraction (%); *Da* = bulk density (0.8 t m^{-3}); *P* = sampling depth (0.20 m); 100 = constant for transformation to t ha^{-1}; and, to determine the total C, the soil C plus biomass C was added [44]

Based on the amount of C stored in each of the crop-growing systems, the amount of carbon dioxide (CO_2 t ha^{-1}) that has been sequestered can be calculated through applying the following equation:

$$CO_2 = C \times Kr$$

where CO_2 = atmospheric carbon dioxide absorbed; *C* = total carbon stored; and *Kr* = 44/12 (constant) [45].

2.4.3. Coffee Yield

Coffee yield was evaluated from the 9 central plants of the net plot. The physiologically mature fruits were harvested annually and expressed in g $plant^{-1}$. The yield per treatment was then extrapolated to kilograms per hectare. Subsequently, to obtain the kilograms per hectare of dry coffee, a conversion ratio of 4:1 was applied; that is, for 4 kg of cherry coffee, 1 kg of gold coffee was obtained. This conversion factor depends on the soil, climate, altitude, agronomic management, age of the plants, harvest, and post-harvest [46].

2.5. Data Analysis

2.5.1. Multivariate Analysis

A multivariate analysis was put into place by employing R Studio (4.2.3) together with the MDA tool package. Moreover, a principal component analysis (PCA) was performed to identify different plant and soil variables in relation to the different arrangements (*E. poeppigiana* + *Myroxylon balsamum* vs. monoculture). This experimental research was conducted from 2018 to 2022. It was necessary to perform scaling since variables were expressed using different units.

Responses established through PCA according to different years and arrangements were modeled as follows:

$$Y_{ijkl} = \mu + A_i + B_j + T_k + A_{Tik} + \varepsilon(_{ijk})l$$

5 Years = i = (2018, 2019, 2020, 2021, 2022); 3 blocks j = 1, 2, 3; 8 arrangement k = 1 to 8,

where

Y_{ijkl} = the observation at the i-th year, the j-th block for the k-th arrangement;
μ = grand mean;
A_i = random effect of the i-th year;
B_j = random effect of the j-th block;
T_k = fixed effect of the k-th companion crop;
AT_{ik} = interaction effect between the i-th year and the k-th arrangement;
$\varepsilon(_{ijk})l$ = Random experimental error (0, σe^2).

2.5.2. Univariate Analysis

Data analysis was carried out using the SAS 9.4 mixed model procedure. A repeated measures approach was employed for consecutive sampling years using a variance–

covariance structure. This was chosen according to the lowest Akaike's Information Criterion [47]. Interactions and main effects were considered significant at $p < 0.05$. Subsequently, variables were verified for assumptions of normality and the homogeneity of variances based on the plot of residuals vs. predicted values. Transformations were realized as required in accordance with the normality assumption, and these transformations were based on the Box-Cox power transformation series [48]. Finally, the LSD mean separation procedure in the SAS mixed model procedure was used in order to separate least square means, which were then ordered into groups using mean separation and denoted using letters [49].

3. Results

PCA analysis showed that the AFS (*E. poeppigiana* + *Myroxylon balsamum*) provided higher N in the plant biomass when compared to the monoculture (Figure 2), except for the monoculture with intensive organic management. A similar pattern was observed for the C and N in soil, where the AFS showed higher levels of these elements. On the other hand, the Cu content was higher in the monoculture, except for the monoculture with intensive organic management.

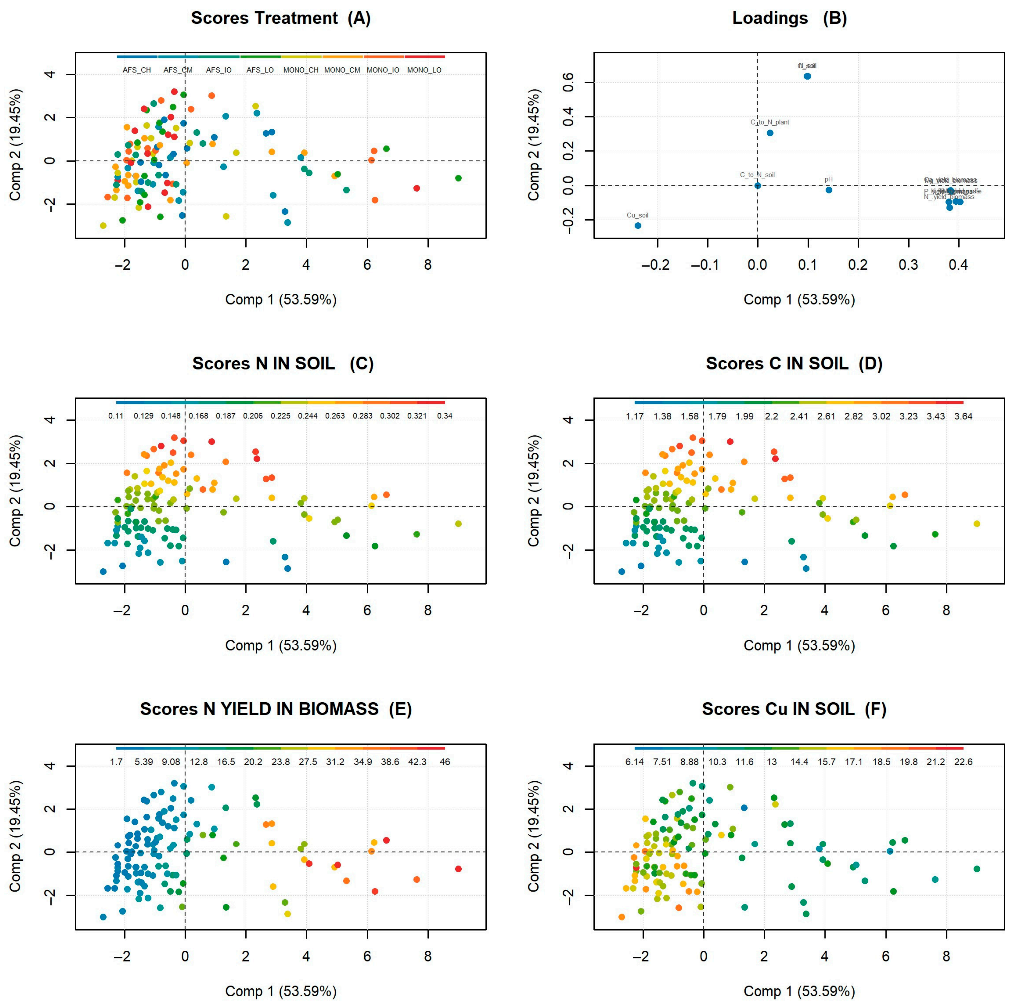

Figure 2. Principal component analysis conducted on coffee plants and related variables. Panel (**B**) depicts the loading, while panels (**A**–**F**) display the score plots. The principal components 1 and 2 are depicted in all figures, showing the influence of different arrangements on different variables from 2018 to 2022. Panels (**C**–**F**) provide the colored score plots, ranging from low to high for the different variables. AFS = *E. poeppigiana* + *Myroxylon balsamum*, MONO is monoculture, CH is conventional high, CM is conventional medium, IO is organic intensive, and LO is low organic.

3.1. Nutrient Concentration in Biomass

The N content contained in the biomass presented statistical differences for the years of evaluation, treatments, and interaction ($p < 0.0001$, 0.02, and 0.01, respectively). The amount of N in the biomass increased from 2018 onward (79%), yet the amount of N was actually lower in 2021 than in 2020 (Table 2).

Table 2. Average values of the N, K, Ca, and Mg content in biomass, determined by the year of evaluation. Within a column and within a given factor, means followed by the same letter are not statistically different ($p < 0.05$).

Year of Evaluation	N (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})
2022	56.6 d	63.6 a	30.4 a	6.2 a
2021	21.4 bc	15.6 b	14.0 b	2.1 b
2020	23.5 c	3.9 d	6.7 d	0.6 d
2019	18.3 b	11.9 bc	10.1 bc	1.2 bc
2018	11.8 a	7.8 cd	6.1 cd	0.8 cd

The biomass N content was greater in the agroforestry system than it was in the monoculture (Table 3). It was determined that the N increment in the systems with *M. balsamum* + *E. poeppigiana* increased independently of the agronomic management. For 2021 and 2022, it was observed that the *M. balsamum* + *E. poeppigiana* system with intensive organic management (36.6 and 83.9 t ha^{-1}, respectively) contributed similar amounts of N to the systems with conventional medium management (40.0 and 78.8 t ha^{-1}, respectively). In 2020 and 2019, the highest N contribution was obtained in the systems with high (44.4 and 48.6 t ha^{-1}) and conventional medium (34.6 and 34.1 t ha^{-1}) agronomic management. Moreover, in 2018, the N contribution was similar to the conventional high (17.4 t ha^{-1}) and intensive organic (16.6 t ha^{-1}) management.

Table 3. Mean values of the N content in biomass, determined by treatment. Within a column and within a given factor, means followed by the same letter are not statistically different (alpha = 0.1).

Treatment	N (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})
M. balsamum + *E. poeppigiana* [1]	40.2 ab	24.6 ab	15.1 ab	2.6 ab
M. balsamum + *E. poeppigiana* [2]	43.5 a	27.3 a	17.5 a	2.9 a
M. balsamum + *E. poeppigian* [3]	42.1 ab	20.2 a	17.8 a	3.3 a
M. balsamum + *E. poeppigiana* [4]	37.0 b	21.7 bc	16.1 a	2.9 a
Monoculture [5]	10.4 c	14.3 de	8.0 c	1.6 bc
Monoculture [6]	14.1 c	12.0 e	7.0 c	1.1 c
Monoculture [7]	11.1 c	17.4 cde	8.5 bc	1.5 bc
Monoculture [8]	12.5 c	18.0 cd	12.7 abc	2.4 abc

Agronomic management: [1,5] conventional high; [2,6] conventional medium; [3,7] intensive organic; [4,8] low organic.

The same behavior was determined in the monoculture; that is, the N content increased over time. In 2022, the highest N contribution was obtained with conventional medium (78.8 t ha^{-1}) and low organic (83.9 t ha^{-1}) management; in 2020 and 2021, the highest N contribution was obtained with conventional high (44.4 and 27.3 t ha^{-1}) and medium (48.6 and 40.8 t ha^{-1}) management. In 2019, the N contribution was similar regardless of the agronomic management (34.61, 34.12, 30.73, and 34.64 t ha^{-1}), and 2018 was the only year where the highest N contribution was achieved with intensive organic (16.6 t ha^{-1}) and low organic (17.4 t ha^{-1}) management (Table 4).

Table 4. Average values of using mean separation the N, K, Ca, and Mg content in biomass, determined by the interaction of year of evaluation and AFS. Within a column and within a given factor, means followed by the same letter are not statistically different ($p < 0.05$).

Year of Evaluation	Treatment	N (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})
2022	*M. balsamum* + *E. poeppigiana* [1]	77.5 a	54.7 c	28.5 bcde	5.5 bc
	M. balsamum + *E. poeppigiana* [2]	78.8 a	83.3 a	37.6 abc	6.8 b
	M. balsamum + *E. poeppigiana* [3]	83.9 a	82.6 a	38.6 ab	7.0 b
	M. balsamum + *E. poeppigiana* [4]	70.1 a	69.2 b	30.9 bcd	6.6 b
2021	*M. balsamum* + *E. poeppigiana* [1]	27.3 efg	17.8 fghijkl	18.5 defghi	3.1 cdefg
	M. balsamum + *E. poeppigiana* [2]	40.8 bcde	27.3 ef	23.1 cdef	4.4 bcd
	M. balsamum + *E. poeppigiana* [3]	36.5 bcde	22.3 fg	22.6 cdefg	3.4 cdef
	M. balsamum + *E. poeppigiana* [4]	31.8 cde	21.6 fhg	18.8 defgh	2.5 defgh
2020	*M. balsamum* + *E. poeppigiana* [1]	44.4 bc	5.3 m	6.2 hijk	1.1 efgh
	M. balsamum + *E. poeppigiana* [2]	48.6 b	5.5 m	4.5 hijk	0.8 efgh
	M. balsamum + *E. poeppigiana* [3]	42.5 bcd	2.9 m	2.8 k	0.5 gh
	M. balsamum + *E. poeppigiana* [4]	39.6 bcde	2.9 m	2.8 k	0.4 gh
2019	*M. balsamum* + *E. poeppigiana* [1]	34.6 bcde	20.3 fghi	18.7 defghi	3.5 cde
	M. balsamum + *E. poeppigiana* [2]	34.1 cde	20.2 fghij	16.3 defghijk	3.4 cdef
	M. balsamum + *E. poeppigiana* [3]	30.7 cdef	18.4 fghijk	15.8 efghijk	3.0 cdefgh
	M. balsamum + *E. poeppigiana* [4]	34.6 bcde	22.3 fg	18.1 defghij	2.7 cdefgh
2018	*M. balsamum* + *E. poeppigiana0* [1]	17.4 fgh	10.3 ghijklm	8.8 fghijk	1.2 efgh
	M. balsamum + *E. poeppigiana* [2]	14.9 ghi	9.8 hijklm	7.6 ghijk	1.0 efgh
	M. balsamum + *E. poeppigiana* [3]	16.6 fghi	10.3 ghijklm	7.7 ghijk	1.0 efgh
	M. balsamum + *E. poeppigiana* [4]	7.8 hi	6.8 klm	5.1 hijk	0.7 efgh
2022	Monoculture [5]	28.9 defg	67.6 b	48.2 a	10.5 a
	Monoculture [6]	40.5 bcde	61.9 bc	26.5 bcde	5.5 bc
	Monoculture [7]	32.6 cde	39.0 de	18.8 defghi	3.5 cde
	Monoculture [8]	40.6 bcde	50.12 cd	14.3 efghijk	4.3 bcd
2021	Monoculture [5]	9.0 hi	10.3 ghijklm	6.8 hijk	0.7 efgh
	Monoculture [6]	14.6 ghi	12.6 ghijklm	7.5 ghijk	0.9 efgh
	Monoculture [7]	6.9 hi	5.0 m	5.6 hijk	0.9 efgh
	Monoculture [8]	4.1 hi	8.0 jklm	8.5 fghijk	1.0 efgh
2020	Monoculture [5]	3.8 hi	4.2 m	3.1 jk	0.3 h
	Monoculture [6]	3.7 hi	3.3 m	1.9 k	0.2 h
	Monoculture [7]	2.5 i	3.6 m	4.1 hijk	0.4 gh
	Monoculture [8]	2.3 i	3.0 m	3.2 jk	0.8 efgh
2019	Monoculture [5]	3.1 hi	3.6 m	2.4 k	0.2 h
	Monoculture [6]	3.5 hi	3.1 m	1.7 k	0.2 h
	Monoculture [7]	3.0 i	3.9 m	4.2 hijk	0.4 gh
	Monoculture [8]	3.1 hi	3.5 m	3.8 ijk	0.8 efgh
2018	Monoculture [5]	6.7 hi	4.4 m	2.8 k	0.3 h
	Monoculture [6]	8.5 hi	5.8 lm	4.7 hijk	0.6 fgh
	Monoculture [7]	10.5 hi	8.7 ijklm	7.3 hijk	0.6 fgh
	Monoculture [8]	12.0 hi	6.2 klm	4.7 hijk	0.8 efgh

Agronomic management: [1,5] conventional high; [2,6] conventional medium; [3,7] intensive organic; [4,8] low organic.

Highly significant differences were determined for the K content in dry biomass for the year of evaluation, treatment, and interaction ($p < 0.001$). In 2022, the highest amount of K (63.6 kg ha^{-1}) was observed (Table 2). The analysis of the treatments showed that the agroforestry system contributed more K than the monocultures (Table 3). The interaction determined that the *M. balsamum* + *E. poeppigiana* system in 2018 with intensive organic management (10.3 kg ha^{-1}) had the highest K contribution, while, in 2019, it was with low organic management (22.3 kg ha^{-1}), in 2020 with conventional medium management (5.5 kg ha^{-1}), and in 2021 and 2022 with conventional medium management (27.3 and 83.3 kg ha^{-1}, respectively). In monocultures, it was determined that, only in 2018 and 2019, low organic management (8.7 and 3.9 kg ha^{-1}, respectively) contributed more K, but in the subsequent years (2020, 2021, and 2022), the highest contribution was obtained in the systems with conventional high management (4.2, 12.7, and 67.6 kg ha^{-1}, respectively) (Table 4). Statistical differences were determined for the Ca contribution per year of evaluation, treatment, and interaction ($p < 0.001$, 0.0017, and 0.0001, respectively). In 2022, the highest Ca contribution was observed (Table 2). Upon analyzing the treatments, it was evident that the AFS contributed more Ca than the monocultures (Table 3). The interaction determined that in 2018 (8.8 t ha^{-1}), 2019 (18.7 t ha^{-1}) and 2020 (6.2 t ha^{-1}), the highest Ca contribution was in the *M. balsamum* + *E. poeppigiana* system with conventional high management. In 2018, the monocultures with the highest Ca contribution were those under low organic management (4.7 t ha^{-1}), followed by intensive organic management (7.3 t ha^{-1}). In 2021, the Ca contribution was highest with conventional medium management and was lowest in monocultures with intensive organic. Only in 2022, in the agroforestry system with intensive organic management (38. 7 t ha^{-1}) and in monocultures with conventional high management (48.2 t ha^{-1}) was there more Ca (Table 4).

Statistical differences were determined for Mg contributions per year of evaluation, treatment, and interaction ($p < 0.001$, 0.0013, and 0.0002, respectively). The highest Mg supply (6.2 kg ha^{-1}) was determined in 2022 (Table 2). Upon analyzing the treatments, it was found that the AFS contributed more Mg than the monocultures (Table 3). The interaction determined that in 2018 and 2021, there was more Mg contribution in the *M. balsamum* + *E. poeppigiana* system with conventional medium management (3.4 and 4.4 kg ha^{-1}, respectively) and in 2019 and 2022 with low organic management (2.5 and 6.6 kg ha^{-1}, respectively) and intensive organic (1.0 and 7.0 kg ha^{-1}), respectively. In monocultures, it was determined that, in the first 4 years, the highest Mg contribution was obtained with low organic management (0.6, 0.4, 0.4, and 0.9 kg ha^{-1}), followed by intensive organic (0.8, 0.8, 0.8, 0.8, and 1.1 kg ha^{-1}), and in 2022, the highest contribution was achieved with conventional high (10.5 kg ha^{-1}) and medium (5.5 kg ha^{-1}) management (Table 4).

3.2. Estimation of Carbon Content

The univariate analysis for carbon present in the soil, C in the biomass, total stored C, and C sequestration in CO_2 equivalents uncovered highly significant differences according to the year of evaluation ($p < 0.0001$). There were no significant differences between treatments and interactions ($p = 0.21$ and 0.10, respectively).

C stored in the soil (2018 to 2022: 30.8, 32.9, 40.3, 46.9, and 37.6 t ha^{-1}) and aboveground biomass (2018 to 2022: 0.24, 0.26, 0.11, 0.43, and 1.26 t ha^{-1}) increased over the 5 years. But in 2022, soil C (37.6 t ha^{-1}) decreased slightly when compared to the previous year (2021: 46.9 t ha^{-1}), and in 2020, there was a decrease in the aboveground biomass C (0.11 t ha^{-1}) with respect to 2019 (0.26 t ha^{-1}) (Table 5. Total C storage (C soil + C biomass) (31.0, 33.1, 40.4, and 47.3 t ha^{-1}, respectively) and C sequestration in CO_2 equivalents (113.7, 121.4, 148.2, and 173.4 t ha^{-1}, respectively) increased over the 5 years, yet, in 2022, a slight decrease in these two parameters is observed with respect to the previous year (Table 5).

3.3. Coffee Yield

Statistical differences were found for treatments, years of evaluation ($p < 0.0001$), and interaction ($p = 0.4154$). It was determined that coffee cultivated with *M. balsamum* + *E. poeppigiana* and monocultures, both with organic management, presented the lowest yields, while the highest yields were obtained in the treatments with conventional management (Table 6). In addition, it was determined that coffee production increased until 2021, after which there was a drastic decrease due to the fact that, in 2022, harvesting only took place in the first 5 months of the year, since the plot was subjected to low pruning in order to regenerate and stimulate tissue (Table 7).

Table 5. Average values of the C content in the soil and biomass, total stored C, and C sequestration in CO_2 equivalents, determined by the year of evaluation. Within a column and within a given factor, means followed by the same letter are not statistically different (alpha = 0.1).

Year of Evaluation	C in Soil (t ha^{-1})	C in Biomass (t ha^{-1})	Total C (t ha^{-1})	CO_2 (t ha^{-1})
2018	30. 8 a	0.24 c	31. 0 a	113.7 a
2019	32.9 a	0.26 c	33.1 a	121.4 a
2020	40.3 b	0.11 d	40.4 b	148.2 b
2021	46.9 c	0.43 b	47.3 c	173.4 c
2022	37.6 b	1.26 a	38.8 b	142.4 b

Table 6. Average values of the coffee yield (kg ha^{-1}), determined by agroforestry systems. Within a column and within a given factor, means followed by the same letter are not statistically different ($p < 0.05$).

Treatment	Yield (kg ha^{-1})
M. balsamum + *E. poeppigiana* [1]	1480.25 a
M. balsamum + *E. poeppigiana* [2]	1599.59 a
M. balsamum + *E. poeppigiana* [3]	682.53 b
M. balsamum + *E. poeppigiana* [4]	689.45 b
Monoculture [5]	1686.39 a
Monoculture [6]	1789.45 a
Monoculture [7]	925.41 b
Monoculture [8]	636.71 b

Agronomic management: [1,5] conventional high; [2,6] conventional medium; [3,7] intensive organic; [4,8] low organic.

Table 7. Average values of the coffee yield (kg ha^{-1}), determined by year of evaluation. Within a column and within a given factor, means followed by the same letter are not statistically different ($p < 0.05$).

Year of Evaluation	Yield (kg ha^{-1})
2018	1102.78 b
2019	1403.91 ab
2020	1452.43 a
2021	1297.11 ab
2022	663.10 c

The interaction determined that the yield in the agroforestry systems was higher in the high and medium conventional management. In 2018 and 2019, the highest yield was achieved with high conventional management (1485.67 and 2071.43 kg ha^{-1}, respectively), and, in the subsequent years, the best yield was obtained with medium conventional management (2631.03, 1898.77, and 800 kg ha^{-1}, respectively). In 2019, 2020, 2021, and 5 months of 2022 with low organic management in agroforestry systems (676.73, 892.93, 1089.33, and 437.3 kg ha^{-1}), it was possible to obtain Robusta coffee yields that were higher than the average reported for the Ecuadorian Amazon (385 to 410 kg ha^{-1}) [50,51]. The yields in monocultures were higher in the years 2018, 2019, and 2022 with high conventional management (2028.53, 2277.4, and 1208.97 kg ha^{-1}, respectively), and with medium conventional management in 2020 and 2021 (2464.5 and 1924.77 kg ha^{-1}) (Table 8).

Table 8. Average values of the coffee yield (kg ha^{-1}), according to the interaction between the year of evaluation and the agroforestry system. Within a column and within a given factor, means followed by the same letter are not statistically different ($p < 0.05$).

Year of Evaluation	Treatment	Yield (kg ha^{-1})
2022	*M. balsamum* + *E. poeppigiana* [1]	779.8 defghijk
	M. balsamum + *E. poeppigiana* [2]	800.0 defghijkl
	M. balsamum + *E. poeppigiana* [3]	304.77 lm
	M. balsamum + *E. poeppigiana* [4]	437.3 m
2021	*M. balsamum* + *E. poeppigiana* [1]	1581.2 cdefgh
	M. balsamum + *E. poeppigiana* [2]	1898.77 abcde
	M. balsamum + *E. poeppigiana* [3]	855.53 ghijklm
	M. balsamum + *E. poeppigiana* [4]	1089.33 ijklm
2020	*M. balsamum* + *E. poeppigiana* [1]	1482.13 cdefgh
	M. balsamum + *E. poeppigiana* [2]	2631.03 ab
	M. balsamum + *E. poeppigiana* [3]	946.7 fghijklm
	M. balsamum + *E. poeppigiana* [4]	892.93 jklm
2019	*M. balsamum* + *E. poeppigiana* [1]	2072.43 abc
	M. balsamum + *E. poeppigiana* [2]	1633.33 abcd
	M. balsamum + *E. poeppigiana* [3]	564.37 cdefghij
	M. balsamum + *E. poeppigiana* [4]	676.73 ghjklm
2018	*M. balsamum* + *E. poeppigiana*[1]	1485.67 abcd
	M. balsamum + *E. poeppigiana*[2]	1034.83 cdefghij
	M. balsamum + *E. poeppigiana*[3]	741.30 efghijklm
	M. balsamum + *E. poeppigiana*[4]	350.93 ijklm
2022	Monoculture [5]	1208.97 ghijklm
	Monoculture [6]	1175.27 ghijklm
	Monoculture [7]	319.17 m
	Monoculture [8]	279.57 klm
2021	Monoculture [5]	1558.97 cdefgh
	Monoculture [6]	1924.77 abcdef
	Monoculture [7]	839.23 ghijklm
	Monoculture [8]	629.1 efghijklm
2020	Monoculture [5]	1555.1 cdefghi
	Monoculture [6]	2464.5 a
	Monoculture [7]	1041.57 ghijklm
	Monoculture [8]	605.5 ghijklm
2019	Monoculture [5]	2277.4 abc
	Monoculture [6]	1965.43 bcdefg
	Monoculture [7]	1333.67 jklm
	Monoculture [8]	999.07 ijklm
2018	Monoculture [5]	2028.53 cdefghi
	Monoculture [6]	1417.3 ghijklm
	Monoculture [7]	1093.4 hijklm
	Monoculture [8]	670.3 klm

Agronomic management: [1,5] conventional high; [2,6] conventional medium; [3,7] intensive organic; [4,8] low organic.

4. Discussion

4.1. Biomass Nutrient Concentration

The N input from legumes increased (from 11.8 kg ha^{-1} in 2018 to 56.6 kg ha^{-1} in 2022). The values in the latter year are higher than some of those obtained over 22 years in an agroforestry system of *Zea mays* and *Sorghum bicolor* (L.) with *Faidherbia albida* in Zambia (34–83 kg ha^{-1}), satisfying between 30 and 71% of the crop N requirements [52]. Thevathasan et al. [53] indicate that when biomass is produced in an agroforestry system, nutrient cycling can, over time, promote the accumulation of N in the soil. Schroth [54] mentions that coffee and cocoa plantations under leguminous shade provide 3 and 14 t ha^{-1} yr^{-1} of biomass, containing 60 and 340 kg of N, while, in alley cropping with perennial herbaceous and woody crops in rotation (*Quercus rubra* and *Carya illinoinensis*), the amount of N in the biomass was 86, 233, 6.3, and 1.9 kg ha^{-1} [55]. In this study, it was determined that AFSs provided a higher N supply than monocultures, which is in agreement with Schroth [54], who mentions that the amount of biomass produced by N-fixing trees has higher concentrations of this element than that produced by non-fixing species, where the amount of N depends mainly on the phenological stage (senescence or fruiting). If the trees are deciduous, they will have a higher N concentration, while, if they are evergreen, they will have a lower N concentration. Consequently, as cocoa is considered a perennial species, the N concentration was lower. Vargas-Tierras et al. [5] mention that the biomass of leguminous species incorporates significant amounts of nutrients. Fahad et al. [52] state that the management of N-fixing trees, commonly called fertilizer trees, in AFSs can be helpful in maintaining crop yields and nutrient cycling, as well as in reinstating soil fertility and conserving soil organic carbon.

Variation in the N supply in conventionally and organically managed plots may occur because of the limitations in the rate of N release from biomass deposited on the soil surface, which is generally influenced by the lignin/nitrogen ratio and polyphenol content within tree foliage [56]. Likewise, another study determined that the N content was lower in an alley system that was 8 years old than in a monoculture [57]. In addition, studies have shown that the N content of biomass is utilized by the crop in the first or subsequent stages of the crop cycle [58].

The increased supply of N, K, Ca, and Mg in AFSs has a direct relationship with the frequency of pruning and mulching, and, over time, this activity will become an effective management practice to reduce farmers' reliance on mineral fertilizers [1]. The contribution of N, K, Ca, and Mg as a function of agronomic management varied greatly between conventional and organic management. The contents obtained in this study were lower than those reported by Montenegro and Romero López [16,59] in AFSs of Arabica coffee with *E. poeppigiana* shade trees more than 10 years old, where the contributions of N, K, Ca, and Mg were 74.9, 46.1, 21.7, and 5.8 t ha^{-1} with conventional high management, 360.0, 205.2, 115.2, and 23.4 t ha^{-1} with conventional medium, and 300.1, 186.3, 96.9, and 18.8 t ha^{-1} with organic medium management. K tends to reach higher values with organic management in AFSs than in organic monocultures [60]. Mg was higher in AFSs than in monocultures; however, the lowest Mg content was obtained in organic AFSs. This behavior was different from that determined by Niether et al. [60] in an 11-year-old AFS of *T. cacao*, where the K content was not affected by the production system and high values were reached in organic systems, while Mg was lower in AFSs than in monocultures. However, when analyzing the Mg content in organic AFSs, the behavior was similar to that of our study.

Finally, it can be inferred that the biomass of the accompanying species in the AFS provides important quantities of N, K, Ca, and Mg, nutrients that coffee needs to achieve maximum vegetative and reproductive growth [61]. Bezerra et al. [62] and Ramírez-Builes and Küsters [21] point out that *C. canephora* demands more Ca and K when the bean is filling out. Rodríguez et al. [23] maintain that the most extracted nutrient in coffee fruits is K, followed by N. Schmidt et al. [23] indicate that the nutrients required by coffee in order of importance are as follows: N > K > Ca > Mg. Therefore, this study affirms that the nutrients required by the main crop can be supplied by the accompanying species that are present in the AFS. Likewise, some studies have revealed that AFSs can contribute to a reduction in farmers' use of chemical fertilizers, thereby ensuring that agricultural systems remain sustainable [63].

4.2. Estimation of Carbon Content

The amount of carbon (C) in the soil at a 20 cm depth increased by 25% with respect to the initial value after five years of evaluation, indicating a significant increase in carbon storage in this period. The analysis of soil C storage showed an increase of 1.3 times from 2018 to 2022. The values found in this study are all above 30 t ha^{-1}, a value that is within the range of C contents determined in AFSs of cocoa and coffee in Indonesia [64], Nicaragua, Honduras, Guatemala, Costa Rica, Costa Rica, and Panama [65]. The total C stored generally increased over the 5 years of evaluation. In 2020, 2021, and 2022, the C storage was 40.4, 47.3, and 38.8 t ha^{-1}, respectively. These contents exceed the C stored in systems of *Coffea* sp. with *Citrus limon* (26.85 t ha^{-1}), *Coffea* sp. with *Macadamia* sp., *I. vera*, *Musa x paradisiaca* L. (34.0, 29.0, and 27.0 t ha^{-1}) [26], and *Coffea* sp. with *Acacia pennatula* (38.47 t ha^{-1}) [66], but are lower than *Coffea* sp. systems with *Inga* sp. (45.42 t ha^{-1}) [67]. Also, the total C stored in 2021 was higher (47.3 t ha^{-1}) than in 2022. This decrease is highly dependent on the biomass input on the soil surface [68], the content and decomposition of organic material [69], and the rate of addition and decomposition. In our study, organic matter at a soil depth of 20 cm was 5.33% in 2021, decreasing to 4.8% in 2022; for this reason, there was an apparent decrease in the C storage content in the final year.

C sequestration in CO_2 equivalents increased from 113.7 to 142.4 t ha^{-1} during the evaluation period in agroforestry systems with *M. balsamum* + *E. poeppigiana*. These values are lower than those reported in AFSs of *Coffea* spp. with *Gliricidia sepium* and *Coffea* spp. and the intercropping of cocoa and coffee with *G. sepium* (231.25 and 198.44 t ha^{-1}) [70]. It is important to note that C sequestration in CO_2 equivalents in 2020 (148.2 t ha^{-1}), 2021 (173.4 t ha^{-1}), and 2022 (142.4 t ha^{-1}) exceeded the C sequestration obtained in a *Coffea* spp. system associated with *Syzygium aromaticum* (137.44 t ha^{-1}) [70]. Finally, the amount of C sequestered in each compartment (aboveground parts, soil, roots, and living biomass) varies greatly depending on several factors, such as the ecoregion or climate, the type of agricultural system (the components' characteristics and the perennial plants' ages, such as that of trees), the quality of the site, and prior land use [71].

4.3. Coffee Yield

The coffee yield was 1112.96 kg ha^{-1} in the agroforestry systems and 1259.49 kg ha^{-1} in the monoculture with the different types of management. These yields are similar (1200 kg ha^{-1}) to those reported by Kouadio et al. [72] in shade-grown Robusta coffee within a tropical monsoon environment in Kontum, Vietnam. But they are inferior to the yields of organic coffee grown under agroforestry systems in Kampala, Uganda (2932.6 kg ha^{-1}) [73]. This yield variation is possibly due to environmental conditions, crop management, and the fact that there is a wide genetic variability of Robusta coffee worldwide [74–76]. These findings allow one to infer that the shade of the companion species in the AFS did not affect the coffee yield. These same results were found by Byrareddy et al., Ehrenbergerová et al., and Le et al. [22,25,77] in several studies where they concluded that shade does not affect the yield. Nonetheless, some studies show that the best yields are obtained when coffee is shaded from 23 to 38%; for example, Arabica coffee under shade has a higher yield (419 kg ha^{-1}) than coffee grown in full sunlight (259 kg ha^{-1}). On the other hand, Le et al. [77] point out that mature coffee plants could have a restricted capacity to adapt to changes in microclimatic conditions generated by shade trees; that is, certain protective benefits derived from shade trees could favor young coffee plants, but not be effective for more mature plants.

The difference in the yield between conventional and organic management is possibly due to the fact that the organic fertilization was not sufficient to meet the nutrients needed in each season, as mentioned by Byrareddy et al. [22]. For this reason, it is advisable to promote the combined use of chemical and organic fertilizers in order to provide valuable guidelines on coffee management practices in the Ecuadorian Amazon, because the excessive use of chemical fertilizers can increase soil acidity, reduce beneficial microorganisms, and accumulate other plant nutrients that can lead to a reduction in yield and production. In contrast, the use of organic fertilizers improves soil texture, creates a favorable environment for microorganisms, and improves water uptake and retention and the efficient use of nutrients [75,78].

In several countries, agroforestry systems stand out as a promising option to promote rural development, alleviating pressure on the soil by integrating multiple uses in the same area and contributing to the preservation of natural resources. However, establishing effective agroforestry systems represents a considerable challenge. In view of this situation, the Central Experimental Station of the Amazon is dedicated to studying agroforestry systems with coffee with the objective of providing insights into the behavior of the main crop when it is cultivated in sustainable production systems.

5. Conclusions

The biomass of the accompanying species in the AFS provided significant amounts of N (79%), K (88%), Ca (80%), and Mg (87%). Although this contribution was not sufficient for coffee grown under agroforestry systems to surpass the yield of coffee grown in monocultures, it did achieve a similar productivity, which is positive since the implementation of AFSs has an environmental benefit.

Dry grain yields in the agroforestry and monoculture systems with conventional and organic agronomic management were similar. In both the AFSs and monocultures, the highest yields were obtained with the medium conventional management; this means that when more nutrients are applied, as in the high conventional management, the plant does not use them to improve its production. On the other hand, when organic management was applied, lower yields were obtained in both AFSs and monocultures; however, additional benefits should be considered, such as less soil, water, and environmental contamination. Based on the above, the implementation of a combined agronomic management (chemical and organic fertilizers) will allow the implementation of sustainable agriculture for producers in the Ecuadorian Amazon. Stored C increased by 18% in soil and 81% in aboveground biomass; overall, AFSs stored and sequestered 20% of C in 5-year-old coffee production AFSs. Therefore, these systems could be considered as an important long-term C reserve.

Finally, the results of this study could be considered a reference for the Ecuadorian Amazon due to the fact that AFSs in our region have helped to guarantee the following: soil conservation, which is undoubtedly the most important aspect; erosion control through soil support in tree roots; the formation of a permanent cover on the surface of the soil caused by the constant addition of biomass; an increase or preservation of soil fertility; and environmental gains, such as C storage and sequestration.

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