

Article

Differences in Manioc Diversity Among Five Ethnic Groups of the Colombian Amazon

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Abstract: Manioc is an important root crop in the tropics and the most important staple food in the Amazon. Manioc is diverse but its diversity has not yet been clearly associated with environmental or social factors. Our study evaluates how variation in edaphic environments and in social factors influences manioc diversity among five ethnic groups of the Amazon region of Colombia. Inventories of landraces, genetic analysis of manioc diversity, visits to farmers' swiddens and interviews with farmers were carried out during two years of field work. Morphotypic and genotypic diversity of manioc were large. The different ethnic groups of our study cultivate different sweet and bitter manioc landraces which they select and maintain in accordance with their ancestral rules and norms. Differences in available environments among indigenous communities (such as the presence of different soils) did not markedly affect manioc morphotypic or genotypic diversity, while social factors considerably influenced observed manioc diversity. Manioc diversity was explained by two parallel processes of manioc diversification: volunteer seedling selection and manioc seed

exchange. We argue that, for a full understanding of manioc diversity, indigenous knowledge, as well as morphological and genetic variation should be taken into account.

Keywords: Amazon; indigenous farmers; manioc diversity; manioc classification; morphological diversity; genetic diversity; volunteer seedling; germplasm exchange

1. Introduction

Manioc (*Manihot esculenta* Crantz), a crop domesticated in Central Amazonia around 8000–10,000 years ago [1], is currently the sixth most important crop in tropical and sub-tropical areas of Africa, Asia and America [2]. The main world collection of manioc is located at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia with more than 5000 accessions. CIAT classifies and conserves this publicly accessible manioc germplasm with an aim to improve crop performance, increase yields under different conditions, and adapt the crop to global climate change [3].

World crop collections maintain a core set of germplasm which is considered the minimum number of specimens that represent the genetic diversity of a crop and its relatives [4]. However, in the case of manioc, core collections apparently do not represent the diversity present in highly diverse regions such as the Amazon region [5–7] and therefore do not necessarily represent the complete diversity of this crop. A way to improve the selection of specimens for their conservation from areas such as the Amazon region is by understanding how manioc diversity is generated and preserved there and what factors affect its diversification. This information is partially available from previous studies conducted in particular areas [6,8,9] or among particular ethnic groups of the Amazon Basin [10,11], but comparison of results between different locations or ethnic groups has so far not been attempted.

The general consensus among manioc geneticists is that manioc diversity is complex and varies depending on whether or not it is assessed by the number of genotypes or morphotypes [6]. A genotype is a specific arrangement of genes that confer morphological and physiological characteristics to a particular group of plants. A morphotype is the outcome of the genetic expression that results in a particular arrangement of morphological features that can be recognized in a group of plants. In the field, populations of autochthonous manioc morphotypes (product of natural and artificial selection) are denominated landraces [12,13]. At least three reasons can be given why the number of genotypes or morphotypes distinguished in a sample differs. One is that manioc is highly heterozygous (bi-allelic state for most important variable sites). It is monoecious (separate male and female reproductive organs on the same plant) and out crossing rates are thus high, making it difficult to interpret manioc variability [14]. A second reason is that the next-generation molecular markers (with high discrimination power) have become available only recently, making it possible to improve the discrimination of genotypes with respect to the number of morphotypes assessed [15]. A third reason is that manioc, although commonly reproduced clonally, also maintains the capacity to reproduce sexually, thus producing seedlings from a recombination of clonally maintained landraces and causing new genotypes that continuously emerge—genotypes that could (or could not) heretofore be distinguished as different manioc landraces [16]. In this paper, we call these seedlings volunteer seedlings [17].

Manioc is the most important staple crop in the Amazon region and exhibits a particularly high diversity there. Manioc diversity is distributed unevenly across different Amazonian environments [18,19] and ethnic groups [10,11,20]. On alluvial soils from the Madeira River (Brazil), for example, a higher manioc genetic diversity was observed than on highly weathered soils or anthropogenic soils of that region [18]. This difference was partially attributed, on the one hand, to the presence of diversity of soil types apt for manioc cultivation. On the other hand, the distribution of manioc diversity has been explained by the indigenous farmers' practice of collecting naturally occurring volunteer seedlings [20] and of cropping, maintaining and exchanging different manioc landraces [8]. Volunteer seedlings are incorporated in farmers' portfolios, thus increasing the number of landraces each farmer holds [17], but also increasing the genetic diversity by inbreeding [21,22]. The selection and maintenance of volunteer seedlings in farmers' portfolios have been attributed mainly to farmers' practices through directional selection in accordance to the agronomic performance of the volunteer seedling and through ideotypic selection when the volunteer seedling is similar to a landrace a farmer already recognizes [21]. However, since volunteer seedlings initially produce a single root [23] and therefore are less productive than manioc propagated clonally, it is not clear how farmers select and why they maintain volunteer seedlings to propagate them clonally. Manioc exchange usually occurs among kin and reflects a particular farmer's social network [10]; sometimes, however, key individuals specialize in the breeding and maintenance of manioc germplasm—as is the case of Amuesha shamans of the Peruvian Amazon [11]. New landraces increase farmers' manioc portfolios; these in turn are exchanged with other farmers—in this way increasing the number of landraces at community level.

Amazonian societies consider manioc to be much more than only a crop, and attribute to it a variety of symbolic meanings that are part of their wider cultural patrimony [8]. For the Piaroa ethnic group of the Venezuelan Amazon, for example, manioc is considered cultural heritage and is used as a mediator of social relationships [20]. The social aspects of manioc might thus encourage or restrict its exchange among ethnic groups and in turn affect manioc diversity of a specific area or region.

With the aim to contribute to the understanding of manioc genotypic and morphotypic diversity in the Amazon region, interdisciplinary research was carried out to study manioc diversity at the community level, indicating what factors affect manioc diversity, how manioc diversity at community level contributes to manioc diversity at regional level and, finally, what information associated to manioc landraces must be considered to select and conserve manioc diversity *in situ* or *ex situ*. The main question guiding our research was: How is manioc diversity shaped by edaphic and social differences among indigenous communities of the Colombian Amazon? To answer this question, manioc morphological and molecular diversity was evaluated in areas inhabited by different ethnic groups. Soil differences and social factors were analyzed to assess their importance for manioc diversification.

2. Results

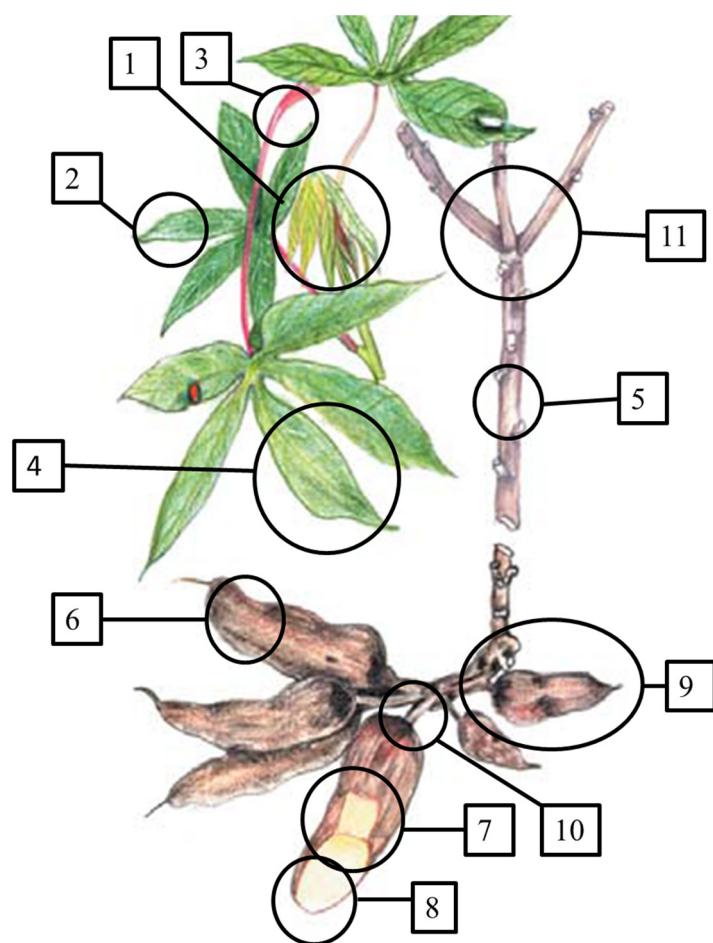
2.1. Manioc Diversity in the Study Area

2.1.1. Morphotypic Manioc Diversity

Indigenous descriptions of manioc landraces were based on homolog morphological parameters comparable to those proposed by CIAT and EMBRAPA (*cf.* Figure 1 and Table S1) as colors and shapes

are morphological characteristics easily recognizable by manioc farmers around the world. All farmers recognized volunteer seedlings as plants that do not grow from stem cuttings, produce only a single bulking root, and appear spontaneously in swiddens on soils where manioc had been grown previously. When CIAT's and EMBRAPA's morphological features for manioc description were discussed with indigenous farmers, they disagreed that the presence/absence of a root stem (Figure 1) might be used as a parameter for manioc classification.

Figure 1. Morphological features used to describe manioc morphotypes. The illustration is a modification (with permission of the Sinchi Institute) of one of the drawings elaborated by Luis Angel Ramos del Águila describing a Tikuna manioc landrace [24].



1) Color of apical leaf ; 2) Color of mature leaf 3) Color of petiole; 4) Shape of the leaf lobule; 5) External color of the stalk; 6) External color of bulking root; 7) Color of bulking root cortex; 8) Color of bulking root pulp; 9) Shape of the bulking root; 10) Presence of root stem; and 11) Plant branching.

Farmers considered that all manioc landraces had a root stem, and that only volunteer seedlings (which have a single root attached directly to the stalk) lacked this. Although among these ethnic groups the presence or absence of a root stem was not considered an appropriate morphological feature to classify manioc, it has been observed that presence or absence of a root stem provided a relevant morphological characteristic to classify manioc landraces cultivated by Makushi people of Guyana [6].

In addition to morphological characteristics of manioc, indigenous people also considered toxicity and use as important features to classify manioc landraces. All five ethnic groups used morphological,

toxicity and use parameters to classify manioc landraces, but classifications differed in complexity among ethnic groups (Table 1). Tikuna people classified manioc landraces into two main groups (Table 1): Maniocs “to eat” composed of manioc landraces with low root toxicity that can be cooked and eaten directly without a detoxification pre-treatment, and bitter maniocs composed of high toxicity manioc landraces that require a process of detoxification before they can be eaten. On the other hand, Andoke, Muinane, Nonuya, and Uitoto people classified manioc landraces into four main groups (Table 1). One group was composed of non-toxic *Manicuera* landraces used to prepare their traditional drink called *Manicuera* (a very sweet juice prepared after grating manioc roots and cooking the squeezed juice in water).

Table 1. Local manioc classification and diversity (number of landraces and between brackets percentage within a community) in five indigenous communities of the Colombian Amazon. T = Total number of manioc landraces per class. EI = Number of manioc landraces associated to ethnic identity in each class; NE = Number of manioc landraces not associated to ethnic identity in each class.

Maniocinventory San Martín de Amacayacu (Tikuna)			Manioc inventory communities “People of the Center”						Total
			Aduche (Andoke)		Guacamayo (Uitoto)	Peña Roja (Nonuya)	Villazul (Muinane)		
Maniocs “to eat”	Total	23 (70%)	Manicuera	Total	2 (6%)	3 (9%)	2 (4%)	2 (7%)	60
				EI	2	3	2	21 ¹	
				NE	0	0	0	0	
	Maniocs “to eat”	Total	9 (28%)	8 (23%)	4 (9%)	7 (27%)			
		EI	8	0	0	1 ¹			
		NE	1	8	4	6			
Bitter maniocs	Total	10 (30%)	Maniocs “to grate”	Total	18 (57%)	14 (40%)	25 (54%)	16 (59%)	113
				EI	14	0	5 ²	2 ¹	
				NE	4	14	20	14	
	Yellow bitter maniocs	Total	3 (9%)	10 (28%)	15 (33%)	2 (7%)			
		EI	3	1	2 ²	1 ¹			
		NE	0	9	13	1			
Total		33			32	35	46	27	173

¹ Of these one *Manicuera*, one manioc “to eat”, one manioc “to grate”, and one yellow bitter manioc were identified by informants as landraces originating from the Bora ethnic group. ² Of these two maniocs “to grate” and one yellow bitter manioc were identified by informants as landraces originating from the Miraña ethnic group.

The second group was composed of low toxicity “to eat” landraces with white or very pale yellowish roots. The third group was composed of high toxicity “to grate” landraces with white to very pale yellowish roots usually used to obtain starch through grating. The fourth group was composed of high toxicity landraces with clearly yellow roots used for different recipes.

From the 173 manioc samples collected and described, 165 came from clonal stems while eight were volunteer seedlings. Among the 173 samples 60 landraces were recognized by local people as non-toxic maniocs which included “to eat” and *Manicuera* maniocs (35%) with low toxicity and 113 as toxic maniocs which included “to grate” and yellow bitter maniocs (65%) with high toxicity. Manioc inventories included between 27 and 46 different manioc landraces per community (Table 1). The average number of manioc landraces per swidden was 12, without significant differences among indigenous communities.

After a detailed review with local farmers of samples from their own community and samples collected across all communities of the Middle Caquetá region, only three duplicates were identified by them.

Differences among communities in the total number of manioc landraces were not significant (chi-square value = 4.5, d.f. = 4; $\alpha = 0.05$), but there were significant differences in the number of low toxicity and high toxicity manioc landraces cultivated among ethnic groups (chi-square values of 10.99 and 21.19, respectively, for low toxicity and high toxicity landraces; d.f. = 4; $\alpha = 0.05$). The Tikuna community maintained more low toxicity manioc landraces than the *People of the Center* communities. On the other hand, the *People of the Center* communities maintained more high-toxicity manioc landraces (Table 1). Although the Tikuna do not themselves make the distinction between bitter manioc landraces with different color of bulking root pulp, we observed that, from the 10 bitter manioc landraces they maintained, eight were yellow bitter manioc and one had pink root pulp.

When the number of manioc “to eat”, manioc “to grate”, and yellow bitter manioc landraces were compared among indigenous communities of the *People of the Center*, significant differences in the number of landraces “to eat” and yellow bitter manioc landraces were found (chi-square values of 10.5 for landraces “to eat” and 25.8 for yellow bitter manioc landraces, respectively; d.f. = 4; $\alpha = 0.05$).

2.1.2. Genotypic Manioc Diversity

Most of the single nucleotide polymorphisms (SNPs) used discriminated manioc samples well after 28 cycles, and only few samples required 33 cycles to obtain proper discrimination. On average observed heterozygosity was 0.39 across the 93 SNPs, with values between 0.04 and 0.69. Around 20% of SNPs had an observed heterozygosity below 0.30 (Table S2).

From the 173 collected manioc landraces considered unique by farmers 44 samples were duplicates, *i.e.*, genetically not 173 but 160 genotypes were distinguished (Table 2). These duplicates were partly collected in different communities with different names and characteristics (30 manioc landraces) and partly collected in the same community (14 manioc landraces).

Morphological comparison of genotype duplicates indicated that different morphotypes could have identical genotypes based on genetic analyses (see duplicate 16, ADU3 and ADU4 in Figure S1). Duplicates could be considered different in one or more features (Table 2), but the manioc class in which the landraces were classified (Z-table value of 0.999), the color of bulking root pulp (Z-table value of 0.974), and the uses (Z-table value of 0.939) were the most common characteristics the duplicates shared.

When the 173 manioc morphotypes collected during our research were compared genetically with manioc genotypes from the CIAT collection, three of the morphotypes collected that corresponded to one genotype (duplicate 2 in Table 2) matched to one of the CIAT genotypes. The remaining 41 duplicates (or 20 genotypes) and the 129 unique manioc morphotypes were genomic material that was new and different from the CIAT core collection, in fact largely grouping into a different cluster (Figure S2). When the genomes of morphotypes collected in this project were compared with genotypes from the CIAT core collection, manioc genotypes from the Tikuna community of San Martín de Amacayacu (AMA) appeared to be closely related to Peruvian manioc genotypes (PER). Manioc genotypes from the communities of the *People of the Center* formed a cluster apart from the bulk of the genotypes provided by CIAT (Figure S2).

Table 2. Comparison of genotypic duplicates indicating which morphological variables indigenous farmers considered equal (value = 1), and which morphological variables they considered different (value = 0) between samples. A Z-test was used to establish the differences among variables discriminating between duplicates. Variables: 1. Class of manioc; 2. Color of bulking root pulp; 3. Uses; 4. Soil in which the landrace grows better; 5. Preferred character to maintain the landrace; 6. Color of petiole; 7. Color of mature leaf; 8. Plant branching; 9. Color of apical leaf; 10. Color of bulking root cortex; 11. Shape of leaf lobule; 12. Root shape.

Variable	1	2	3	4	5	6	7	8	9	10	11	12
z-value obtained	3.27	1.96	1.52	1.09	1.09	−0.21	−0.65	−1.09	−1.52	−1.96	−2.40	−2.40
z-value expected (two-tailed 95% confidence interval)	0.99	0.97	0.93	0.87	0.87	0.40	0.25	0.12	0.60	0.02	0.00	0.00
Duplicates	Duplicates among Communities											
1.(ADU10, GUO18, GUO22)	1	0	0	0	0	0	0	0	0	0	0	0
2. (ADU16, GUO6, AMA1)	1	1	1	1	0	1	0	0	0	0	0	0
3. (ADU1, GUO29)	1	1	1	0	1	0	1	0	0	0	1	0
4. (ADU11, GUO13)	1	0	1	0	0	0	1	1	0	0	0	0
5. (ADU18, GUO4)	1	1	0	0	1	0	1	0	0	0	1	0
6. (ADU23, GUO8)	1	1	1	0	0	1	1	1	0	0	1	0
7. (ADU30, GUO10)	1	1	0	1	0	1	0	0	0	0	0	0
8. (AMA28, GUO9)	1	1	0	1	0	1	1	1	0	1	0	0
9. (GUO31, PRO23)	1	1	1	1	1	0	1	0	1	1	0	0
10. (PRO2, VA12)	1	1	1	1	1	1	0	0	1	1	0	1
11. (PRO21, VA24)	1	1	1	1	1	0	1	0	0	0	0	0
12. (PRO40, VA19)	1	1	1	0	1	0	0	0	0	1	0	0
13. (PRO42, VA11)	1	1	1	1	1	1	1	1	1	1	0	0
14. (PRO8, VA23)	0	0	1	0	1	0	0	1	1	0	0	0
Duplicates	Duplicates within Communities											
15. (ADU32, ADU27)	1	1	0	1	0	0	0	1	1	0	0	0
16. (ADU3, ADU4)	1	1	0	1	1	0	1	1	0	0	0	0
17. (AMA7, AMA17)	1	1	1	1	0	1	0	0	1	1	1	1
18. (AMA3, AMA30)	0	0	1	1	1	0	0	0	1	0	1	1
19. (AMA31, AMA32)	1	1	0	1	1	1	0	0	0	0	0	1
20. (PRO9, PRO47)	1	0	1	0	1	1	0	1	0	0	0	0
21. (VA13, VA15)	0	0	1	1	1	1	0	0	0	0	0	1

Codes: ADU: Samples from the Andoke community of Aduche; AMA: Samples from the Tikuna community of San Martín de Amacayacu; GUO: Samples from the Uitoto community of Guacamayo; PRO: Samples from the Nonuya community of Peña Roja; VA: Samples from the Muinane community of Villazul.

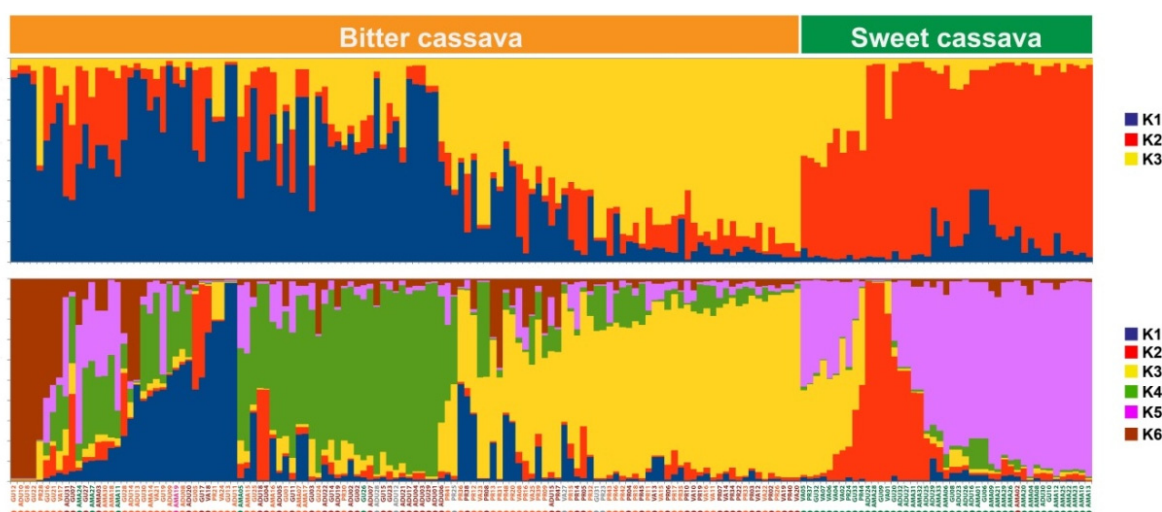
When a genetic analysis of manioc samples was done according to their toxicity, “to eat” manioc formed a cluster apart from bitter manioc (“to grate” and yellow bitter manioc), except for few samples (Figure 2). *Manicuera* manioc on the other hand were genetically more related to high toxicity manioc

than with low toxicity manioc (Figure 2). With $K = 3$ and $K = 6$, one cluster (in red in the first STRUCTURE graph and red and pink in the second STRUCTURE graph in Figure 2 combined most of the “to eat” manioc. Although some of the “bitter” manioc were placed in the “sweet” manioc cluster and *Manicuera* manioc were placed in the “bitter” manioc cluster, indigenous morphotypic and toxicity organization into “to eat” and bitter manioc corresponded largely with the clustering based on genomic information.

Figure 2. Genetic distribution of manioc landraces according to their toxicity. (A) Neighbor-Joining dendrogram showing the genetic relation of the different manioc types identified by indigenous people; (B) STRUCTURE output of the molecular diversity of sampled manioc according to their toxicity as sweet (low toxicity) and bitter (high toxicity) manioc obtained with the most likely number of clusters ($K = 3$ and $K = 6$). Each vertical colored bar represents one genotype and the colors indicate the extent to which a genotype genetically belonged to the clusters.



Figure 2. Cont.



(B)

From a total of 173 samples, 74% had high genomic variability indicating they were the product of mixing of several genomes. The remaining 26% of the samples had low genomic variability with single population membership coefficients (Q) higher than 0.9. The proportions of landraces with high/low genomic variation were comparable across communities: 73%/27% for San Martín de Amacayacu, 72%/28% for Aduche, 77%/23% for Guacamayo, 78%/22% for Peña Roja, and 81%/19% for Villazul.

2.2. Sources of Manioc Landraces

2.2.1. Mythical Accounts of the Origin of Manioc Use

Ethnic groups from the Amazon region of Colombia consider manioc as a native species. Manioc domestication is attributed to mythical beings and this knowledge is passed on orally from generation to generation.

In Tikuna narratives, manioc came from the mythic “tree of abundance”. The maintenance and preparation of this mythic manioc were in the hands of an ancient woman and her husband, a bird that was the owner of fire [25]. Manioc was the food that allowed the mythic twins Yoi and Ipi to procreate and feed their off-spring. According to these narratives, the origin of the Tikuna was directly related to manioc: when Yoi went fishing, he used different baits to fish different animals, but when he used a piece of sweet manioc to fish at the Eware River, he fished the Tikunas [26]. That is why the Tikunas know how to cultivate sweet manioc—their main food.

According to narratives of the *People of the Center*, manioc existed before humans as a Paempa tree (Paempa is the name Andoke people use to refer to *Manicuera* manioc). According to Y.A., one of the students of Yiñeko, the Andoke leader that rebuilt Aduche after the rubber boom, “the first *Manicuera* landrace as we know it today originated from a branch of the Paempa tree that fell down and stuck into the ground” (Interview with Y.A., November 22, 2012). All the ethnic groups of the *People of the Center* agree that *Manicuera* was the first manioc. Andoke people consider it their initial food. In Muinane words, *Manicuera* is “the breast of Mother Earth and our first food”. An Andoke mythical tale recalls that “after The Flood, DidanAikA, the wife of the Andoke God also identified as the Mother Earth, took

branches of the *Paempa* tree and cut them into small sticks. From each stick originated a new manioc landrace” (Interview with F.A., August 15, 2012).

All *People of the Center* received *Manicuera* but each particular ethnic group received different manioc landraces. This manioc distribution was further explained to us through a mythical tale shared by all *People of the Center*, and told by an elder Uitoto woman:

“When God started to distribute the manioc landraces among the *People of the Center*, he first distributed *Manicuera* among all groups. Then he distributed manioc “to grate” to Andoke, Muniane and Bora people. It was getting late and there were still other groups waiting for manioc. He finally gave to Uitoto and Nonuya people yellow bitter manioc. Because it was too late to grate them, Uitoto and Nonuya women put the roots into the water. That is why Uitoto and Nonuya women don’t know how to grate manioc” (Interview with V.M., April 25, 2013).

The Andoke also recount that after Nenefi, the Andoke God, gave them the manioc stalks, he recommended people to search for a burnt, soft soil called *Ñesxaθ* to cultivate them. In the case of the Uitoto, they were instructed to plant manioc on *Juk+no* soils. *Ñesxaθ* and *Juk+no* are Andoke and Uitoto terms for Amazonian Dark Earths (ADE) [27]. The use of ADE for manioc cultivation is also referred in Muinane history. According to an elder woman who was present at the establishment of Villazul at the beginning of the 20th century: “the community was located on this black soil [ADE]. The first swiddens were done there [on ADE] because those soils are good for manioc growth” (Interview with A.K., November 27, 2012).

2.2.2. Sources of Today’s Manioc Inventories

According to the indigenous people participating in our study, the manioc landraces they presently cultivate come from three different sources: landraces received in mythical times (and that provide them with their distinct ethnic identity); landraces obtained throughout their history and which furnish clans with their particular identity; and landraces obtained through exchange with farmers from outside the community.

Mythical narratives indicate that some manioc landraces were given to indigenous people as ethnic identity markers (*Manicuera* for example), yet these are not all the manioc landraces that today bestow an ethnic group with its identity. Tikuna and Uitoto people for example share a common historical event in which other manioc landraces appeared at the time clan differentiation within the ethnic group took place. Tikuna narratives point to a time when individuals were undifferentiated and nobody knew who their relatives were. To avoid this situation, the Tikuna started to use different manioc landraces to create differences between clans (families not bonded by consanguinity). Uitoto people specify that clan differentiation strengthened their identity by selecting particular landraces. According to Uitoto narratives, before a clan was formed people prayed asking for manioc landraces that could grant them identity as a clan. Then they searched for new manioc landraces from those that grew spontaneously (volunteer seedlings) and selected some as their own.

In addition to the manioc landraces that people maintained as ethnic identity markers, other landraces were obtained through exchange. According to our respondents, from the 173 manioc landraces collected

in our study, 116 (67%) were obtained through exchange (mainly with neighboring communities), but some landraces came from very distant places—including places outside the Amazon Basin (11% of the total inventory of manioc morphotypes). The remaining 57 landraces (33% of the total inventory of manioc morphotypes) were manioc landraces identified by indigenous farmers as landraces that characterized them as members of a particular ethnic group (including mythical and clan-specific landraces). These 57 landraces also included four Bora manioc landraces introduced by a Bora woman living in Villazul as well as three Miraña landraces introduced by a Miraña woman living in Peña Roja. These landraces are now part of the manioc portfolio of the Muinane and the Nonuya communities respectively (see Table 1).

When these landraces that bestow ethnic identity were analyzed genetically, 100% of the *Manicuera*, 53% of manioc “to eat”, and 40% of yellow bitter landraces turned out to be genotypes with low genomic variability and thus with a high single population membership or Q-value (Figure 3). For landraces without a specific ethnic association, the percentage of genotypes with low genomic variability was between 10% and 30%. None of the “to grate” manioc had a Q-value higher than 0.9, but 43% of the manioc “to grate” with ethnic specificity had a Q-value higher than 0.8 (Figure 3).

When patterns of manioc planting in swiddens were evaluated, the most frequent patterns did not prevent manioc sexual recombination between toxic and non-toxic varieties or within manioc groups (Figure 4). In swiddens manioc landraces are commonly organized randomly or grouped in patches according to manioc group, so manioc landraces are never truly separated from others. Therefore, each swidden creates opportunities for crossing among manioc landraces.

Figure 3. Genomic variability as expressed by the single population membership coefficient or Q-value of manioc landraces collected in five indigenous communities of the Colombian Amazon region; a distinction is made between landraces grouped according to ethnic classification (*cf.* Table 1) and whether or not they are with/without ethnic specificity.

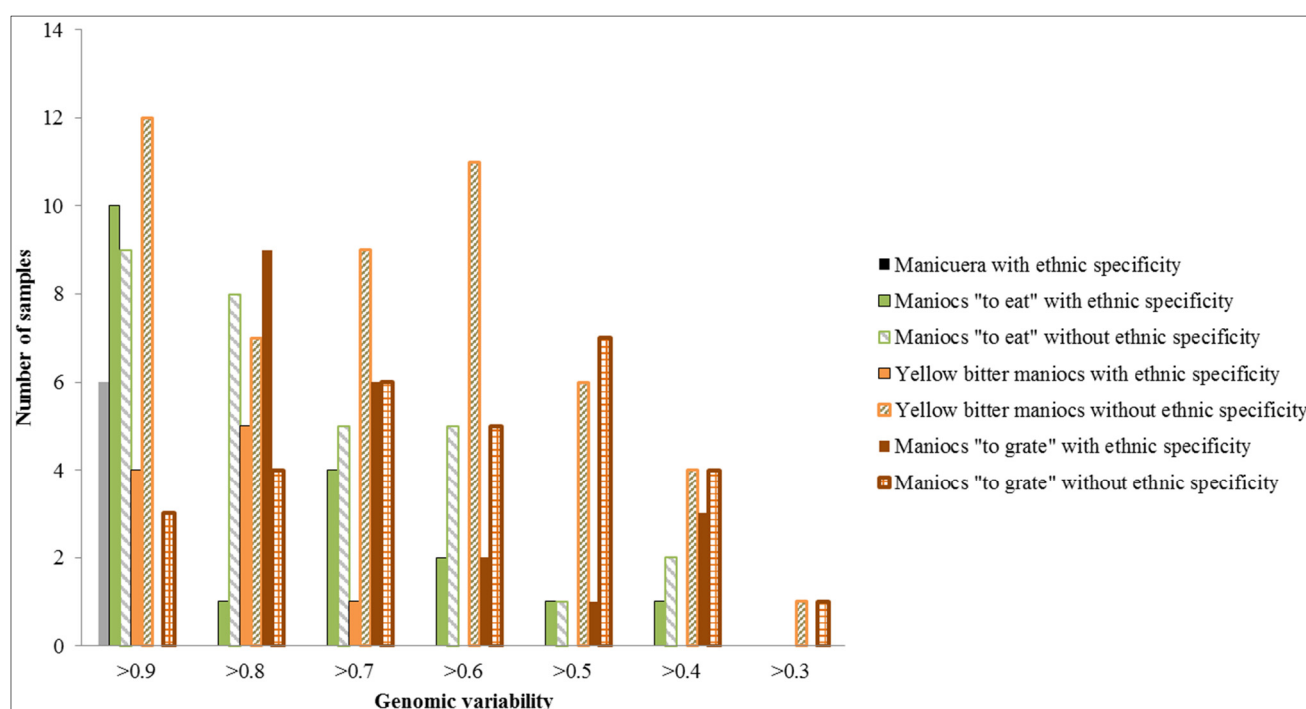
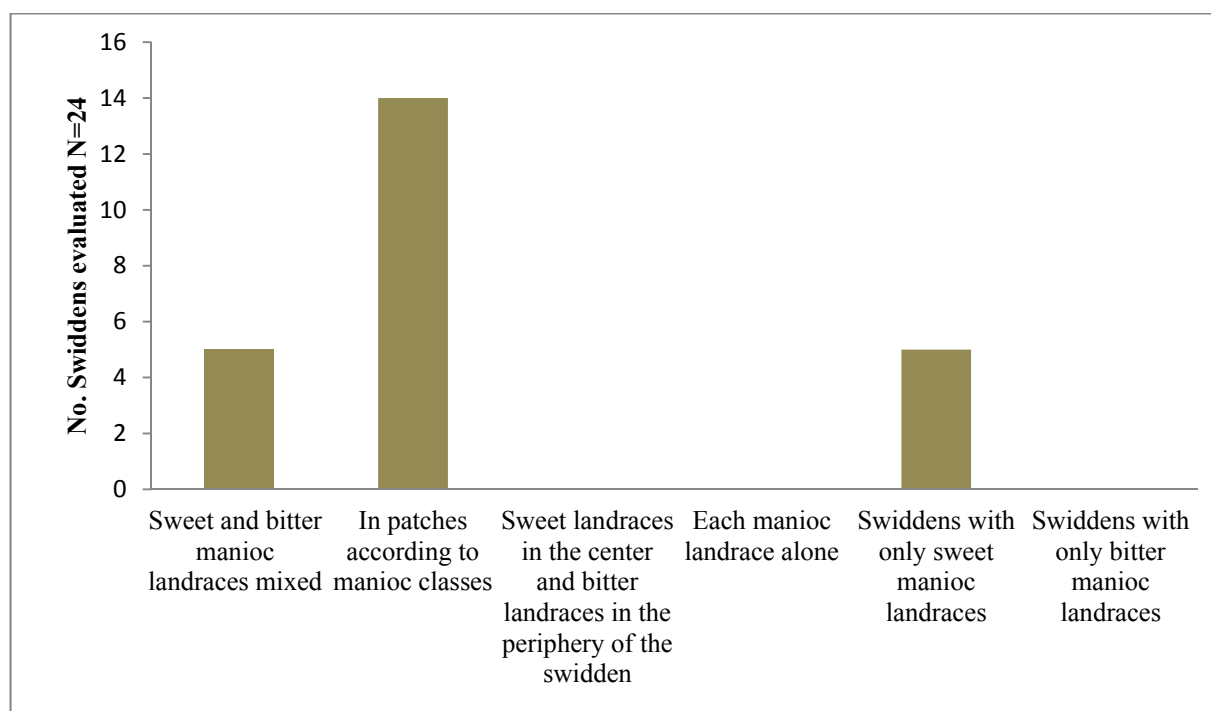


Figure 4. Manioc planting patterns in swiddens of indigenous communities.

Note: The pattern of sweet landraces in the center and bitter landraces in the periphery of the swidden was reported earlier by Van der Hammen for swiddens located in the Middle Caquetá River region [28] and also observed elsewhere by McKey and Beckerman [29].

2.3. The Effect of Different Soil Environments on Manioc Diversity

From the five communities studied, San Martín de Amacayacu was the only one without access to ADE. However, as mentioned above, there were no significant differences in the number of manioc landraces between communities, so the presence of ADE did not increase the number of landraces in communities of the Middle Caquetá region. Moreover, from the 173 manioc landraces indigenous people identified, 119 (70%) were classified by them as landraces that grow well in any kind of soil. Among manioc landraces that, according to our respondents, performed better in highly weathered soils (six landraces), alluvial soils (19 landraces) or ADE (29 landraces) there were landraces from all the four different classes. In the cases in which indigenous farmers indicated that some landraces grew better in highly weathered soils, these landraces were not cultivated in ADE; we were also told that these same landraces did not tolerate soils with a high moisture content and the roots rotted easily when cultivated in floodplains. In the case of landraces that performed better in alluvial soils, our respondents indicated that these landraces quickly developed bulking roots and could be harvested early (one manioc landrace from San Martín de Amacayacu, for example, takes 3 months on average to be ready for harvesting). In the case of landraces that performed better in ADE, farmers indicated that these landraces produce big roots rich in starch.

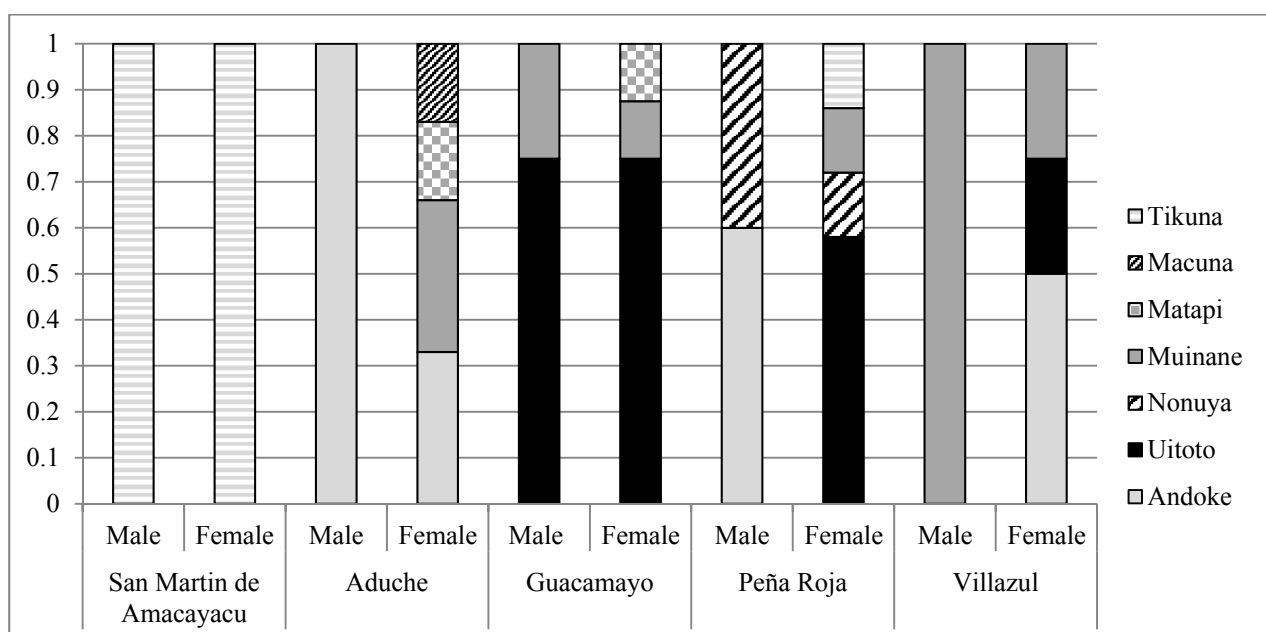
2.4. The Effect of Manioc Exchange on Manioc Diversity

Indigenous farmers readily exchange manioc landraces on different occasions. Important exchanges occur primarily between mothers, grandmothers and daughters when the latter get married. Because all

communities studied are patrilocal, when a woman marries, she leaves her community and/or home and goes to live at her husband's community and/or home, adopting her husband's ethnic traditions. Before she leaves a cross-generational exchange of manioc landraces occurs, and the newly-wed receives landraces from her mother and grandmother as part of her dowry. As soon as she arrives to her husband's community/home, her mother-in-law introduces her to the husband's traditions. One of the first activities the new couple has to do is to open a swidden to produce their own food. At that moment, the mother-in-law gives her daughter-in-law the manioc landraces that are specific to her son's ethnic group. She will plant them, but mix them with the manioc landraces given to her by her family. Over time, she will exchange with her mother-in-law (and other women in her husband's community) her own manioc landraces, increasing in this way family manioc inventories and, when partners come from different communities, community manioc inventories.

Traditionally, in most indigenous groups of the Amazon region, men were not allowed to marry women from other ethnic groups. Because of this, manioc stocks of different ethnic groups could develop into distinct clusters. During the last century, however, marriage rules have considerably changed. In the case of the *People of the Center*, for example, interethnic marriages offered patrilocal groups in danger of extinction a chance for cultural survival (Figure 5). Nowadays, the ethnic composition of *People of the Center* communities is a mix of ethnic groups but where variation is always larger for women than for men. These changes are reflected in the arrangement of genotypes of manioc landraces in which there are no clear clusters formed by a community (Figure 2). In the case of San Martín de Amacayacu, in which most of the marriages are between Tikuna partners (in fact, in our sample all the couples were 100% Tikuna; see Figure 5), manioc landraces form a more compact cluster than the landraces of the *People of the Center*.

Figure 5. Ethnic composition (in fractions) of the group of family farmers in each community who participated in this project.



Apart from manioc exchanges mediated by marriages, opportunities for manioc exchange that are not marriage dependent also occur. Often, women who travel outside the region bring back with them landraces from the places visited (for example, the landrace GUO27 in Figure S2 which was brought in from the Vaupes region in the northeast of the Colombian Amazon). This eagerness to increase manioc inventories was also apparent during our collective visits to swiddens to describe and classify manioc landraces. Farmer visitors commonly asked a swidden's owner for stalks of varieties they did not yet possess. This was also observed during collective manioc harvesting of swiddens. The practice of free manioc exchange is part of being an indigenous farmer. In fact, from an indigenous point of view a good farmer is one who maintains a high number of different landraces (and not necessarily the one who produces more per unit of land). In our case, the farmer families that each community selected to participate (*i.e.*, the most appreciated farmers) in the study were also those with the most diverse swiddens (in terms of the number of landraces held by each family).

2.5. Indigenous Culinary Traditions

According to indigenous mythical tales, since not all manioc landraces were equally distributed over all ethnic groups, their culinary traditions also differed (Table 3). The composition of manioc inventories of each community therefore, also reflects the culinary traditions of each ethnic group.

Seventy percent of the manioc landraces found in San Martín de Amacayacu were “*to eat*” landraces. Tikuna culinary tradition is therefore based on sweet manioc mainly. People from San Martín de Amacayacu affirmed that they did not possess bitter manioc until recently—about 50 years ago or so, when yellow *farinha* was first prepared for sale. They also stated that they traditionally knew the technique to prepare *farinha* from sweet manioc landraces and that they still prepare it for household use, but that they produce yellow *farinha* for cash. *People of the Center* cultivate *Manicuera* landraces exclusively and indeed *Manicuera* landraces grouped together—with the exception of one sample (VA27) which is a *Manicuera* landrace of the Bora ethnic group. They use *Manicuera* landraces to prepare their traditional drink (*Manicuera*) which in their mythical tales is considered as their food and identity symbol. Excluding *Manicuera*, each particular ethnic group recognized other manioc landraces as their own. The Uitoto and Nonuya communities of Guacamayo and Peña Roja held the highest number of yellow bitter manioc landraces. At the same time, these ethnic groups claimed that recipes elaborated with yellow bitter manioc were their own traditional preparations (Table 3). The *farinha* that is commonly associated with the use of yellow bitter manioc was not a traditional preparation of Uitoto and Nonuya people. According to them, they learned how to prepare *farinha* from their yellow bitter manioc during the rubber boom—when this storable product was used as the main food to feed indigenous slaves. Andoke and Muinane people on the other hand claim to have the tradition to use manioc “*to grate*” and claim that *caguana* and starch *casabe* are traditional preparations of theirs.

Table 3. Traditional ethnic recipes based on manioc where A stands for Andoke, M for Muinane, N for Nonuya, T for Tikuna and U for Uitoto. Recipes known by different ethnic groups but which are not exclusive to any one group are identified with an X.

Ethnic Preparations	A	M	N	T	U
Based on <i>Manicuera</i> maniocs					
<i>Manicuera</i> (Manioc grated and boiled for a sweet manioc juice)	A	M	N		U
Based on maniocs “to eat”					
Arapata (Manioc cooked and mixed with banana)				T	
Colada (Manioc starch cooked with water and sugar)				X	
Dry casabe (Round bread made from the manioc root)				X	
<i>Farinha</i> (A granulate of fermented and roasted manioc)				T	
Jutiroi (Juice of fermented manioc leaves boiled)					U
Manioc juice boiled with fish	X	X	X		X
Masato (Manioc beer obtained from a mix of mashed manioc and sweet potatoes)				T	
Monegú (Boiled manioc and kneaded with fish)				T	
Payavarú (Boiled manioc, mixed with toasted manioc leaves and squeezed)				T	
Payavarú wine (Fermented Payavarú)				T	
Pururuca (Masato with banana)				T	
Starch casabe (Round bread made of manioc starch)				X	
Tapioca (A granulate of toasted manioc starch)				T	
Unchará (Manioc bread)				T	
Based on maniocs “to grate”					
Arepa (Baked round bread)	X	X	X		X
Caguana (Boiled starch and mixed with fruit juice)	A	M	X		X
Colada				X	
<i>Farinha</i>	X	X	X		
Manioc juice boiled with fish	X	X	X		X
Starch casabe	A	M			
Tamal (Manioc root packed in banana leaves and steamed)	X	X	X		X
Tapioca				X	
Based on yellow bitter manioc					
Arepa	X	X	X		X
Caguana	X	X	N		U
Colada				X	
Dry casabe			N		U
Manioc juice boiled with fish	X	X	X		X
<i>Farinha</i>	X	X	X	X	X
Tucupí (Source made cooking the fermented bitter manioc juice with hot chilies)	X	X	X	X	U
Jukui (Tucupí with fish and/or shrimps)					U
Mingao (<i>farinha</i> mixed and water)					X
Starch casabe				X	
Tapioca				X	
Tamal	X	X	X		X

However, maniocs “to grate” are also commonly used by other ethnic groups and in fact they form the dominant group of manioc landraces in the manioc inventories of indigenous communities of the

Middle Caquetá. The Tikuna are also starting to use bitter manioc to prepare traditional recipes that require starch (*colada* and *tapioca*), thus moving away from their more traditional sweet manioc use.

Against our expectations, when complexity in the preparation of recipes (in terms of number of steps and time required) was taken into account, no direct relation between complexity and toxicity was found. There are Tikuna recipes made of non-toxic manioc that take several days to prepare such as *farinha*, *masato* (manioc beer), *payavarú*, *payavarú* wine, and *tapioca*, and the *People of the Center* use toxic manioc that also take days to prepare such as dry *casabe* and *tucupí*. However, when complexity in terms of the time spent to prepare recipes made of “to grate” or yellow bitter manioc landraces was compared, we found that the preparation of recipes from manioc “to grate” took less time than those prepared with yellow bitter manioc. Manioc “to grate” are used mainly to obtain the starch of the roots by grating and washing them with water to eliminate cyanogenic compounds and precipitate the starch. On the other hand, preparations made of yellow bitter manioc required root fermentation in water to liberate cyanogenic compounds, followed by the grating and squeezing of the root biomass to eliminate the cyanogenic compounds before cooking. Differences in the time required for bitter manioc processing might explain why the use of manioc “to grate” in communities that traditionally use mainly yellow bitter for their dishes is becoming more popular.

On the other hand, and according to the Tikuna, the use of yellow bitter manioc among indigenous communities was promoted by outsiders for the elaboration of *farinha* as a non-perishable cash product, and so were new products indigenous people highly appreciate (such as *tucupí*). In this latter case, the toxicity of bitter manioc is related with other organoleptic characteristics that are perceived by indigenous people as culinary advantage.

Indigenous people know the danger of consuming bitter manioc when not properly prepared, yet they are not concerned about bitter manioc toxicity as they do not perceive this as a limitation to consume them. In fact, what we observed is that, presently, indigenous people from the communities we included in our study increase the use of bitter manioc instead of, as we expected, increasing the use of sweet manioc.

3. Discussion

3.1. Manioc Diversity and Manioc Classification in Indigenous Communities of the Colombian Amazon

A total of 173 manioc landraces were collected and distinguished based on morphological parameters. Manioc diversity in study areas of comparable size ranged between 100 and 204 different morphotypes [10,11,20]. This means that the methodology we used (*i.e.*, to assess community manioc diversity through a small group of experienced farmers) gave results comparable to earlier studies.

The numbers of distinguished morphotypes and genotypes differed in our inventory, as also observed in earlier studies [5,6,30]. In some cases, morphological variations of identical genotypes can be attributed to genotype by environment interactions that cause unique morphotypes of the same genotype which local people classify as different landraces [11]. In other cases, morphologically similar landraces growing together are the result of an ideotypic selection of volunteer seedlings mixed with an already known landrace, resulting in more than one genotype per morphotype [17]. In this study, 87% of the morphotypes indigenous farmers distinguished were also considered genetically different; this indicates

that farmers recognized more variability in the morphological features of the landraces than the genomic assay did. The high coincidence between morphotypic inventories and genotypic inventories indicates that the descriptors used by indigenous farmers to discriminate different manioc landraces were rather accurate, and that they are experts in discriminating landraces in the field. This expertise has been developed through permanent contact with the different manioc landraces in their swiddens and the efficient transmission of this body of knowledge to other women.

The 93 SNPs selected to discriminate manioc genetic diversity allowed to differentiate different locations and communities (Figure 2a) and manioc groups (Figure 2b). The average observed heterozygosity across the 160 genotypes was 0.39, similar to heterozygosity reported for samples from Brazil [31]. Using SNP genotyping to discriminate samples genetically, 22 duplicates of genotypes were obtained among 173 samples. These duplicates matched landraces with morphological differences in one or more features (Table 2). However, most of the duplicates were consistently classified into the same manioc class according to their toxicity and use, or consistently identified by the color of the bulking root pulp. These two variables define the main characteristics indigenous farmers want to see in a landrace and this in the end will define the way in which a given landrace can be used.

Among scientists, manioc variability is classified in different ways, but toxicity (expressed in terms of the content of cyanogenic compounds) is by far the main feature for manioc classification [32,33]. Manioc landraces with less than 100 mg/kg of cyanogenic compounds are classified as sweet or of direct culinary quality, and manioc landraces with more than 100 mg/kg of cyanogenic compounds are classified as bitter or of industrial quality [32,34], implying a major processing step is required. The concept of industrial quality is alien to indigenous classifications but by and large they follow the same major distinction of manioc (Table 1) as needing a major processing step or ready to eat after minor processing. In addition, they add the color aspect as of major (culinary) importance.

Accidents of manioc poisoning are extremely rare in Amazonian indigenous communities; apparently, a biocultural co-evolution between people and manioc occurred [16]. We evidenced no constraints whatsoever in the use of high toxicity manioc among indigenous farmers, even though we observed an increase in the use of high toxicity manioc. The use of low toxicity and high toxicity manioc encourages indigenous farmers to maintain both types of manioc in their production systems and thus a potential source of manioc variability that would, of course, be limited in regions where bitter manioc are not appreciated for human consumption. However, farmers outside the Amazon Basin (as in some African countries) also find advantages in the cultivation of bitter manioc [35,36] and are increasingly including high-toxicity landraces in their productive systems [16].

It has been proposed that sweet and bitter manioc were domesticated in different historical moments, and that these manioc were distributed unevenly throughout the Amazon region—even though they are presently cultivated together in fields [37]. Our results show a clear genetic clustering for both sweet and bitter manioc; this might support arguments in favor of a different origin of these two manioc classes. It has been proposed that sweet manioc landraces were domesticated in what is today the state of Rondônia in Brazil [38] and that from there they spread along the main rivers of the Amazon Basin. This might explain why in Tikuna communities (such as San Martín de Amacayacu) located on the Amazon River and its main tributaries the use of sweet manioc varieties is dominant, while among ethnic groups living in the interfluvial region bitter manioc dominate. Results also indicate the importance of interethnic marriages among neighboring ethnic groups. This implies a relatively high seed exchange

rate between neighboring groups that also exchange brides as compared against seed exchange with far away communities, e.g., between San Martín de Amacayacu and the communities of the Middle Caquetá region. This seed exchange through marriage between neighboring groups has also been observed in other communities [39–42]. The lack of exchange over longer distances strengthens geographical isolation, thus increasing overall manioc diversity in the Amazon region.

When the genomic composition of the morphotypes collected in this study were compared with the genotypes provided by CIAT's world collection most of the samples form a cluster apart from CIAT's genotypes. This indicates that although some of the genomic variability of maniocs from the Colombian Amazon is represented in CIAT's core collection, there is a substantial genetic diversity that is not contained by it. Similar findings were obtained when the germplasm of some Amazonian maniocs was compared with CIAT's core collection using microsatellites [6,7] is far from covering all available diversity. The current study also showed clear that potentially diversity available in Colombian Amazon is poorly represented in CIAT's collection.

3.2. Sources of Manioc Variability among Ethnic Groups

Seed exchange mechanisms are important sources to increase and maintain crop agrobiodiversity in the Amazon [39], and manioc exchange is most likely to occur among nearby communities. However, manioc exchange can reduce diversity as active out-crossing of the different landraces exchanged may homogenize crop diversity [43]. Our dendrograms show that there exist differences in manioc diversity among groups geographically separated by hundreds of kilometers; these differences however also appear in the cluster of the Middle Caquetá region where communities exist relatively close to one another. Delêtre *et al.* [40] found that, in areas where ethno-linguistic boundaries are present, particular manioc groups are formed (and which they attribute to differences in marriage rules and therefore manioc exchange). Notwithstanding the differences in marriage rules between the communities studied by Delêtre *et al.* and the communities we researched (where, until recently, manioc exchange was limited by ethnic boundaries), the effect of marriage rules on manioc diversity seem to be comparable. In our case, social rather than geographical exclusion allowed for the emergence of a relation between particular manioc landraces and ethnic identity; this in turn possibly strengthened the bond within, and the differentiation between, ethnic groups. For indigenous groups of the Colombian Amazon, manioc is not merely a crop; it also constitutes a symbol of identity. There are maniocs that identify ethnic groups with a common origin (*Manicuera* for *People of the Center*) as well as maniocs that identify particular ethnic groups (manioc “to eat” for the Tikuna, yellow bitter manioc for the Nonuya and Uitoto and manioc “to grate” for the Andoke and Muinane). This link between ethnicity and manioc apparently occurs elsewhere as well, as is the case with the Miraña and the Bora (see above) or the Piaroa of the Venezuelan Amazon [20]. This particular segregation of manioc among the different ethnic groups constitutes a source of manioc variation in the region.

Some of the maniocs that provide ethnic groups with their identity must have been obtained from volunteer seedlings selected long ago (so long ago that indigenous people consider them to be of a mythical origin), those that provide clan identity clearly showed that they were obtained through the selection of sporadic volunteer seedlings. These historical moments of volunteer seedlings selection are clear examples of a directional selection of volunteer seedlings. What most probably started as a selection

for agronomic performance [21] could later have led to the selection of a manioc landrace for distinctive features that could help clans to claim it as their own. Volunteer seedlings constitute an important source of manioc variability and are important for the selection of manioc landraces as ethnic identity markers. Today, indigenous farmers continue to incorporate volunteer seedlings into their manioc pools as 5% of the landraces they described were obtained from recently selected volunteer seedlings.

The fact that 33% of the total manioc inventory of landraces constituted a symbol of identity, and that from these *Manicuera*, more than 50% of the “to eat” manioc and 40% of yellow bitter manioc also have low genomic variability indicates that these landraces have been conserved without major modification to their genomes (that is: without significant recombination). The importance (in terms of historical memory) of these landraces for each one of the ethnic groups studied warrants the special treatment they receive. This fact notwithstanding, all manioc landraces (whether they are or are not attached to ethnic identity) are managed in the same way and planted in patterns that do not prevent possible sexual recombination of landraces. In contrast to other ethnic groups in which shamans play an important role in the conservation of manioc landraces, in the communities we studied, women are wholly responsible for the management of manioc inventories and their conservation. Results indicate that indigenous women have a deep knowledge of these landraces and furthermore partake in effective knowledge transfer networks involving young women to perpetuate their conservation. This is why ethnic groups that have production systems based on swidden agriculture, have been less affected by outside interventions, and are still able to pass on the knowledge required to manage, conserve or otherwise increase manioc inventories. Swiddens can be considered effective *in situ* reservoirs of landraces with low genomic variability.

Duputié *et al.* [21] indicated that the incorporation of volunteer seedlings that had a too high level of inbreeding could reduce agronomic quality, while volunteer seedlings that had a too high level of outbreeding could blur highly appreciated landrace features. Apparently, the indigenous people we studied handle these probabilities appropriately through their intimate knowledge of the morphological characteristics that distinguish important landraces from others, by reproducing them clonally, by planting different landraces in clusters to maintain landraces’ most important characteristics, and by testing volunteer seedlings for two or three production cycles before deciding whether to maintain them or not; the latter practice was also observed in the Wayãpi indigenous group [21]. These practices effectively help avoid the negative effects of cross-breeding in clonal populations.

As we show, the use of low toxicity or high toxicity manioc was directly associated to ethnic culinary traditions (and which in turn provided a specific ethnic or clan-specific identity). Wilson and Dufour [44] also found a direct relationship between the manioc inventories of the Tukano of the Colombian Vaupés and their culinary traditions. McKey *et al.* [16] propose that the use of particular manioc landraces among ethnic groups might be the result of the type of society. Accordingly, high toxicity manioc would be most favored in large, sedentary societies along floodplains of major rivers where fish supply is abundant and a good part of the time could be used for high toxicity manioc processing. On the other hand, the use of low toxicity manioc might be favored in smaller and more mobile societies where the main activities are based on hunting and gathering and where there is not enough time for processing manioc or for making or transporting the tools needed for it to a new settlement.

The hypothesis of McKey *et al.* [16] however does not hold for the case of the indigenous communities of this study. *People of the Center* who do have a tradition to use high toxicity manioc do

not originate from the floodplains of major rivers but rather from the interfluvium between the Caquetá and the Putumayo Rivers where the supply of fish is also limited [45]. Although they have moved out of their ancestral territories to areas with fertile floodplains, their tradition to use mainly high toxicity manioc has not changed. On the other hand, the Tikuna, a much less mobile society than the ethnic groups of the Middle Caquetá region, adopted high-toxicity manioc only recently and mainly as a way to obtain some cash needed for goods they cannot themselves produce. We therefore found that the preference for low toxicity or high toxicity manioc was unrelated with the ease of processing and hence, according to our respondents, not an important reason to select or grow landraces.

3.3. Sources of Manioc Variability among Communities

Fraser and Clement [46] and Fraser *et al.* [19] suggested that ADE could constitute a different edaphic environment for manioc cultivation which might drive an artificial selection of manioc landraces that perform better in those soils—thus increasing manioc diversity at the community level. However, the number of manioc landraces in our community inventories did not differ significantly between communities with or without access to ADE. Although according to indigenous farmers a few manioc landraces performed better in certain soils, most of the manioc landraces they cultivate grow well in any soil. For these indigenous communities, a soil-manioc specificity has not been encouraged, moving their manioc stocks indiscriminately from one swidden to the next. Having manioc landraces that easily adapt to soil variations allows farmers to harvest enough manioc to satisfy their requirements independently of the soil characteristics of the swidden. Our respondents showed little interest in increasing manioc yields and seemed more concerned in preserving a variety of landraces to satisfy their culinary traditions, thereby maintaining what they deem to be a good quality of life. Our findings match those of Hastik *et al.* [47] who also found no preference for the use of anthropogenic soils in an Amazonian region with low land use pressure. The favoring of a soil-manioc specificity might be an important aspect in Amazonian societies that are more integrated in the market and more interested in cultivating manioc as a cash crop, showing a proclivity to select specific soils such as ADE in order to improve yields as in the case of *caboclos* from the Madeira River [9].

Although a manioc-soil specificity was not found in the communities we studied, Andoke and Uitoto narratives attribute an important role to ADE in the conservation of the first manioc indigenous people manipulated. This is in agreement with the hypothesis that manioc is a native species of Amazonia [16,48] and that ADE might have played an important role in manioc domestication [38].

One factor that affected manioc diversity at community level was manioc exchange. The common inclination of farmers to maintain high numbers of manioc landraces can be explained through their concept of life-quality which is based on the notion of abundance of landraces and products. For indigenous people, the good life is “to eat well and live well” which basically means continuous access to a variety of foods which in turn will secure good health, harmonious relationships with the family and the other members of the community, as well as a prolific progeny [49]. Essential to this purpose is to secure an abundance of products and landraces that may serve as a buffer in difficult times.

Manioc exchange among indigenous communities of the Middle Caquetá region could be appreciated in the dendrograms of manioc genetic diversity. Dendrograms also showed a close relation between Tikuna manioc landraces and landraces from Peru (Figure S2). This latter relation could be explained

by the geographical position of the community of San Martín de Amacayacu and the distribution of the Tikuna ethnic group in the region: the Tikuna's traditional territory corresponds to the upper part of the Amazon River which includes parts of the Brazilian, Colombian and Peruvian Amazon [50]. For the Tikuna, borders within what they consider to be their territory do not exist, and therefore sharing and exchanging manioc landraces with other Tikuna communities of the three countries is common. Finally, manioc exchange has also been induced by external pull factors such as the recent cultivation of high toxicity landraces to prepare yellow *farinha* as a cash product which is easily sold within the community and in close-by urban centers.

Apparently, instead of an erosion of manioc diversity due to outside interventions, manioc diversity has been maintained in the last century and, in some cases, increased by the interplay of three different factors. First, the continuity of local culture which has encouraged indigenous people to maintain manioc landraces with low genomic variation. Second, the continuous experimentation with volunteer seedlings in the field. Third, the willingness of indigenous farmers to maintain highly diverse inventories of manioc in their swiddens as a symbol of a good quality of life. From our perspective, as long as the relationship between indigenous people and manioc continues, the conservation of a high manioc diversity in the region is possible. In this sense, manioc could be considered more than just a crop in the Amazon region: it also needs to be seen as cultural heritage of Amazonian societies.

However, the permanent threats to the conservation of the Amazon Basin, together with the pressure to integrate indigenous people in currently prevailing economic models severely increases the risk of loss of indigenous crop diversity [11,51]. For this reason, it is expedient to understand the drivers of current manioc diversity and to locate manioc variability hotspots. Information about these issues might be important for those interested in maintain and increase public manioc collections, and for those interested in the conservation of manioc diversity, to help develop strategies to conserve these materials *in situ*. There are already some experiences showing that it is possible to protect both crop diversity and the collective knowledge indigenous people have of crops by way of geographical indications or denominations of origin [52] in which the use of molecular tools such as SNPs to discriminate landraces from specific localities or ethnic groups is accurate [53–55]. Since it is difficult to maintain a real representation of diversity in worldwide crop collections, the possibility to acknowledge and incorporate local farmers in the conservation of diversity could be a real alternative for crop conservation and the preservation of independent cultures. Unfortunately, initiatives to do so are still in their initial stages [56].

4. Conclusions

Inventories of manioc of communities were both morphotypically and genotypically different, with a good correspondence between the two. The high manioc diversity observed in the five indigenous communities of our study is the result of different social factors that have historically affected manioc since its domestication. These social factors have played a more important role than for example e.g., differences in edaphic environments. The main reason for this is that manioc, the staple food for indigenous communities of the Amazon region, is a symbol of identity. Manioc variation is intimately related with ethnicity or clan membership and with local culinary traditions that express this identity. Manioc diversity is therefore linked to cultural aspects and it explains the importance and conservation

of each one of the manioc landraces that today exist in our area of study. These aspects are lost when manioc landraces are conserved outside the communities that shaped these people-manioc relationships.

We sampled only five communities in the Colombian Amazon; since the Amazon Basin is inhabited by hundreds of different ethnic groups—each with its distinct people-manioc relationship—the number of manioc landraces in this Basin can be expected to be high and diverse. The knowledge indigenous farmers and, particularly, women have of the characteristics of the manioc landraces they manage, constitutes an important factor for the conservation of particular manioc landraces with low genomic variability. In addition to the particular manioc landraces conserved by each ethnic group, the incorporation of volunteer seedlings into farmers' portfolios and manioc exchange mechanisms are two important and permanent sources of manioc variability in the region.

Swidden agriculture provides indigenous people the opportunity to continue managing highly diverse stocks of toxic and non-toxic manioc, as well as to select and conserve volunteer seedlings that spontaneously appear in their swiddens. Nevertheless, the production system of the indigenous communities from our study is not any more common place in the Amazon Basin or beyond. Today, many indigenous groups are disappearing or merge into larger societies—to the detriment of cultural diversity [57]. Models of manioc production in which production depends on a limited number of genotypes with clear commercial advantages reduces the possibilities for social mechanisms to act in favor of promoting and sustaining manioc diversity.

The manioc diversity shown in this study represents a small sample as we only studied five of 420 ethnic groups [57]. Nevertheless, this diversity is not fully represented by CIAT's world collection. *In situ* conservation of manioc diversity is possible but this can only be attained when the production systems of indigenous communities are themselves also conserved. It is therefore necessary that institutions interested in manioc conservation develop strategies to recognize and learn from these communities how they created their highly diverse manioc portfolios and help them conserve them as an important legacy for present and future generations.

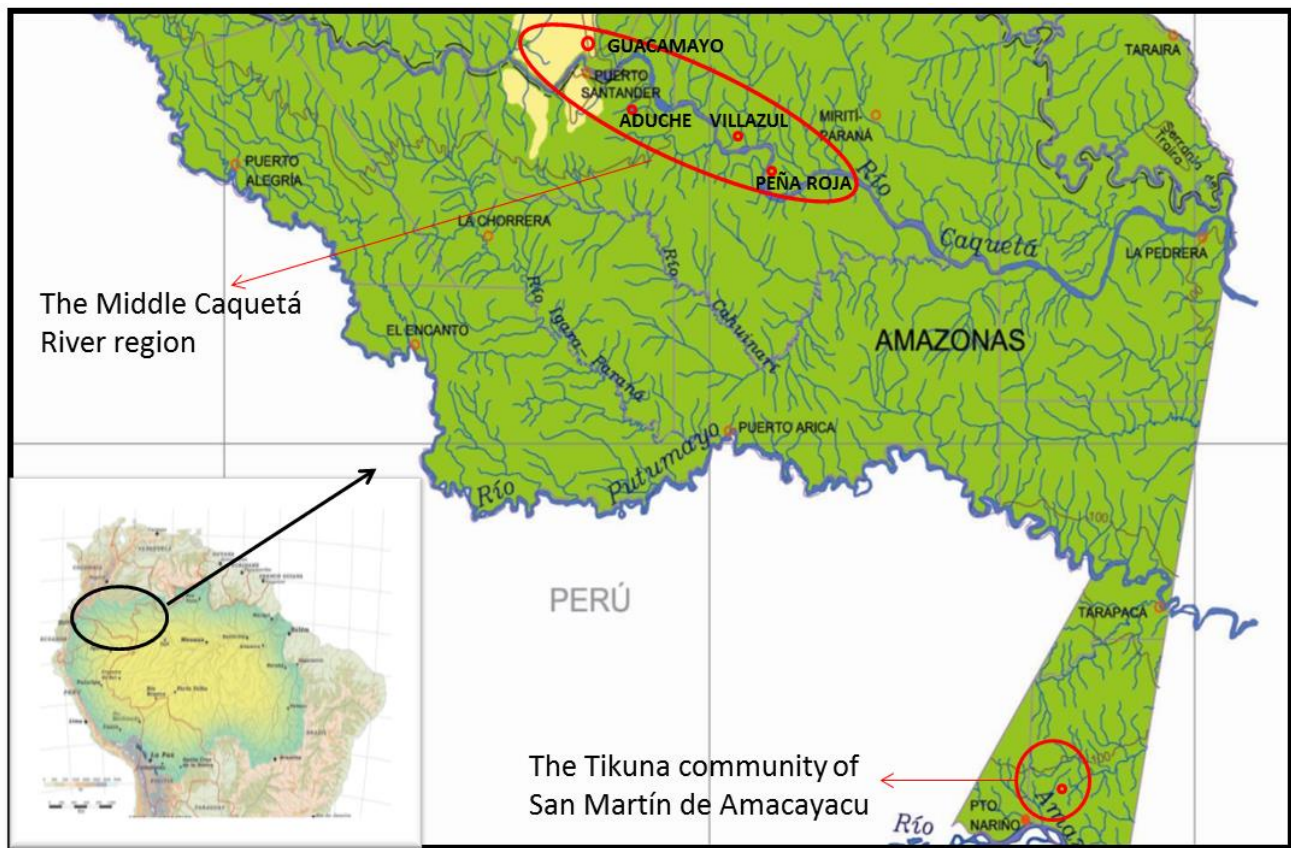
5. Materials and Methods

5.1. Study Area

Throughout our research we studied two elements that affect manioc diversity: soil diversity and socio-cultural arrangements. For the study, we selected the Colombian Amazon region, part of upper Amazonia, where manioc diversity has been poorly studied. In the case of soil diversity we were particularly interested in the way ADE affects manioc cultivation, as the use of these soils by indigenous people has been poorly documented. For this, and with an aim to compare two locations with contrasting soils, we selected an area in the southern part of the Colombian Amazon in which ADE has not been reported, and an area in the Middle Caquetá River in which ADE has been reported. To assess the relation between socio-cultural aspects and manioc diversity we selected the most representative ethnic groups and the most traditional communities of each area of study. Accordingly, research was carried out among five ethnic groups (Figure 6). The first are the Tikuna living in the community of San Martín de Amacayacu, located in the lower section of the Amacayacu River, a tributary of the Amazon River. A second cluster of four ethnic groups (the Andoke community of Aduche, the Uitoto community of

Guacamayo, the Muinane community of Villazul, and the Nonuya community of Peña Roja) is located in the Middle Caquetá River region.

Figure 6. Map showing the two study areas where research was carried out. The map is based on the hydrographic map of the Amazon region (Sinchi 2002; scale 1:500.000).



San Martín de Amacayacu ($03^{\circ}50'17.3''$ South and $70^{\circ}17'57.3''$ West) lies within the limits of the Amacayacu National Park and is part of the Tikuna, Cocama and Yagua Indigenous Reserve (TICOYA) which covers approximately 140,000 ha. The area is characterized by flat plains of ancient, low alluvial terraces with superficial soils as a result of the high phreatic level of the area. Elevation is around 100 m above sea level with slopes between 1%–3%. The average annual rainfall is 2800 mm. The area is covered by a mature dense forest most in a mature stage with abundant epiphytes [58] with moderate anthropic intervention.

The area of the Middle Caquetá River embraces two indigenous reserves of which the communities of Aduche, Guacamayo, Villazul and Peña Roja are a part. The Aduche Indigenous Reserve (approximately 62,000 ha) includes the communities of Aduche ($00^{\circ}39'21''$ South and $72^{\circ}17'32''$ West) and Guacamayo ($00^{\circ}31'25''$ South and $72^{\circ}22'38''$ West) and the Nonuya Indigenous Reserve (approximately 59,840 ha) includes the communities of Villazul ($00^{\circ}40'00''$ South and $72^{\circ}16'32''$ West) and Peña Roja ($00^{\circ}44'29''$ South and $72^{\circ}05'09''$ West). The area is formed by the intersection of sedimentary plains of Tertiary origin with rocky outcrops of Paleozoic origin, creating elevated plateaus. The area also includes alluvial plains of the Caquetá River and its tributaries. Elevation ranges between 200 and 300 m above sea level with slopes between 7%–25%. The average annual rainfall is 3000 mm [59].

The area is covered by a mosaic of vegetation types ranging from mature dense forest to small herbs and bromeliads on the rocky formations [58], with moderate anthropic intervention.

All the indigenous communities that participated in this research project have access to highly weathered soils and alluvial soils for manioc production [60–62]. Highly weathered soils classified as Oxisols and Ultisols [63] are acid (pH 3–5.5) soils, characterized by prolonged periods of weathering and leaching during their formation, resulting in soils with a low cation exchange capacity, limited amounts of cations and a very low phosphorous availability. Alluvial soils (classified as Alluvial Entisols) originate from the annually enriched Andisols sediments from the flooding of the Caquetá and Amazon Rivers and their tributaries [64].

The community of San Martín de Amacayacu is the only one without Amazonian Dark Earth (ADE). ADE are very fertile soils with good physical and chemical properties and very suitable for agriculture [65,66]. They hold large amounts of organic matter and are less acidic than the Oxisols or Ultisols from which they were formed, resulting in a better cation exchange capacity and base saturation compared to background soils [67]. ADE not only have more available nitrogen, calcium, and phosphorous [68] but are also less affected by leaching, resulting in a longer-term nutrient availability as compared to background soils [69].

5.2. Fieldwork

The research we undertook was based on a free, prior informed consent agreement between the Instituto Amazónico de Investigaciones Científicas Sinchi and the communities associated to the indigenous organization Asociación de Comunidades Indígenas del Trapecio Amazónico (ACITAM) which includes the community of San Martín de Amacayacu and the indigenous organization Consejo Regional Indígena del Medio Amazonas (CRIMA—which includes the communities of the Middle Caquetá region) to work together on all aspects of traditional food production (Agreement between Sinchi Institute and the AZICATCH, CRIMA and ACITAM indigenous organizations of June 2004) [70]. The activities carried out also obeyed the two main missions of the Sinchi Institute [71]: First, to support the Ministry of Environment in carrying out their commitments and the development of activities stemming from Colombia's participation in international treaties and agreements (such as its support to article 8j of the Convention on Biological Diversity); second, to encourage the development and dissemination of knowledge, values and technologies related to the management of natural resources of ethnic groups of the Colombian Amazon through participatory action research.

Field work was carried out between September 2011 and September 2013. Each community was visited eight times for two to four days, for a total of 20 days per community. For a better understanding of manioc diversity, both morphological and genetic diversity was estimated. Morphological diversity was assessed in the field together with indigenous farmers in 2011, while genetic diversity of the landraces that were recognized by indigenous people was estimated in the laboratory in December 2013. Cultural elements associated to manioc diversity were recorded in all communities between 2011 and 2013. All the information obtained was discussed and analyzed by the authors of this paper.

5.3. Populations

Five ethnic groups of the Colombian Amazon region participated in this research: Tikuna, Andoke, Muinane, Nonuya and Uitoto.

The Tikuna people are originally from the upper Amazon Basin; their language (Tikuna) stems from an independent linguistic family. Tikuna people can be divided into two main groups of clans, those with feathers and those without feathers; marriages generally only occur between members of opposed clans, thus promoting exogamy [72]. During the rubber boom at the beginning of 1900s, the Tikuna were less affected than the ethnic groups from the Middle Caquetá region, as the former apparently offered less resistance to slavery than other groups. At present the Tikuna number approximately 35,500 individuals distributed across the Amazon region of Brazil (71%), Colombia (23%) and Peru (6%) [73]. The community of San Martín de Amacayacu is inhabited by 440 inhabitants (153 families; community census of 2011) who live on one side of the Amacayacu River. Despite having had long contact with white people throughout their recent history, the community of San Martín de Amacayacu maintains its traditional culture.

Uitoto, Andoke, Muinane and Nonuya people presently living in the Middle Caquetá region identify themselves as ethnic groups with a common geographic origin encompassing the area between the Putumayo and the Caquetá rivers. Because of this they call themselves “*Gente de centro*” (*People of the Center*). Therefore, Uitoto, Andoke, Muinane and Nonuya share different cultural aspects such as the common origin of their languages (rooted in the Wuitoto linguistic family), the construction and use of *malokas* (large houses where traditional leaders are consulted and where they pass on their knowledge) and the use of the coca leaves and tobacco as elements required to establish dialogue within the *malokas*. Between 1908 and 1909 Thomas Whiffen visited the area between the Putumayo and the Caquetá rivers and estimated the number of Uitoto at 15,000, of Andoke at 10,000, of Muinane at 2000 and of Nonuya at 1000 [74].

The population of *People of the Center* was strongly diminished by the rubber boom. In some cases (e.g., the Nonuya and Muinane) only few men survived. Therefore, these groups broke with the tradition to marry only between clans of their own ethnic group and made new arrangements to marry women of other ethnic groups (O.P., personal communication). This allowed them to increase their numbers and perpetuate their particular cultures—at the expense of creating multiethnic communities. The present Uitoto population is estimated to be 8400 inhabitants, with 77% living in Colombia and 23% in Peru where they arrived as slaves during the rubber boom. According to the 2011 community census, the Uitoto community of Guacamayo consists of 153 persons (34 families). Aduche is the only Andoke community that exists today. This community is located on Andoke ancestral territory and inhabited by 128 (27 families). The community of Villazul is one of two Muinane communities that remains today. The community of Villazul is inhabited by 77 persons (17 families). The community of Peña Roja is the only Nonuya community that exists today. It is inhabited by 71 persons (15 families).

After explaining the participatory approach of the project and the activities our research would consist of each community selected what they considered to be the most experienced and active farmer families to work with us in this project. Twenty families from San Martín de Amacayacu (13% of the population), ten from Guacamayo (29% of the population), nine from Aduche (33% of the population), eight from Villazul (47% of the population), and six from Peña Roja (40% of the population) were selected. The selected families were composed of couples or elder people and their daughters or sons. Because not all

the families were of the same ethnic origin, information about the ethnicity of men and women was recorded. Because women are directly responsible for planting and cooking manioc, it was also assessed whether they followed the cultural traditions of their husbands or their own traditions when cultivating or cooking manioc.

5.4. Ethnobotanical Data

5.4.1. Manioc Inventories

An inventory of manioc landraces managed by each community was made to determine manioc morphotypic diversity. Morphotypic diversity was understood as the number of different manioc morphotypes conserved per community and across communities. With each of the communities separately an inventory of their manioc landraces was made to determine manioc diversity. At the start, a preliminary list of the names of the manioc landraces present in the community was elaborated through a group discussion with women farmers. This was followed by visits to swiddens starting with the swiddens local people considered to be the most diverse. In the field, the farmers discussed which plant represented which landrace and jointly a description was made. Whenever new landraces were found, these would be added to the list. When no more new landraces were found on a swidden, the group went to the next swidden to look for other landraces. This procedure was repeated until the people indicated there were no further manioc landraces in their community. This ensured that most landraces present in the village were recorded, although there is a possibility that some landraces may have been left out from these inventories. The same procedure was repeated in all communities.

The description of manioc landraces was based on morphological characteristics used by indigenous people of the communities and made them equivalent to the morphological indices developed by CIAT [75] and EMBRAPA [76]. The descriptors of manioc plants included: apical leaf color, mature leaf color, leaf branch color, shape of the leaf lobule, color of the stalk exterior, color of the bulking root cortex, color of the bulking root pulp, shape of the bulking root, presence of a root stem, and architecture of the plant (Figure 1); a photographic record of each landrace was taken. The overlap between the descriptors used by indigenous farmers and the descriptors used by CIAT and EMBRAPA is included in Table S1 of supplementary material and the complete morphologic description and photographic record of each manioc landrace recorded in this study is provided in Figure S1 of supplementary material.

Complementary information collected on manioc landraces included: common name, traditional name, type of manioc according to indigenous classification, type of soil in which the landrace grows best, origin of the landrace (sexual when the landrace was recognized by the farmer as a volunteer seedling or propagated from a volunteer seedling; clonal when the farmer obtained the landrace from a stem cutting), location from where the landrace was obtained, and its uses. Complementary information was used to assess volunteer seedling frequency in indigenous swiddens and their management by farmers.

In order to triangulate the individual inventories of each community and to look for duplicates, after completing the community inventories group discussions were held with farmers who participated in the description of the manioc landraces. To this purpose, the pictures and the description of each landrace were used.

5.4.2. Inventory of Ethnic Manioc Dishes

Women from the different ethnic groups were interviewed to list all recipes they knew in which manioc was the main ingredient and to assess the frequency with which those recipes were prepared. They were asked about all types of preparations including fermented and non-fermented drinks, main meals, snacks, condiments and any preparation they considered were important to include in the list. In this way an inventory of recipes was obtained. The research team often helped in the preparation of meals and to share these with members of the communities; these were important opportunities to observe culinary traditions and learn about the manioc landraces frequently used in them.

5.5. Genetic Data

From each one of the landraces described by indigenous people in the field, one to three samples of apical sprouts were collected in paper bags with silica gel as dehydrating agent. Upon arrival at the laboratories of the Sinchi institute in Leticia (Colombia) the samples were completely dried with silica gel and preserved at -20°C .

Plant molecular analyses were carried out within the framework of the new legislation for research institutes associated with Colombia's Ministry of Environment (Decreto 1376 of 2013) [77], and in which the Sinchi institute does not need permission for genetic resources assessment when the material is collected without a commercial interest and for research purposes only.

The samples were processed in the Manioc Genetics Laboratory of CIAT, Palmira (Colombia). Upon arrival the samples were lyophilized overnight using an Alpha 2-4 LDplus Martin Christ Freeze-dryer (Germany). From lyophilized samples DNA was extracted using Qiagen (Venlo, Netherlands) DNeasy Plant 96-well extraction kits.

Genetic diversity of manioc has generally been assessed by the study of single sequence repeats (SSRs). However, only a limited number of SSR markers are polymorphic, limiting the power to assess genetic variability in manioc [53]. On the other hand, single nucleotide polymorphisms (SNPs) are the most abundant type of DNA polymorphisms in eukaryotic genomes. For manioc, one SNP can be found per 121 nucleotides. A total of about 2954 SNPs have been found for manioc from which 1190 have been technically and biologically validated for manioc [15] making them much more abundant than SSRs [53]. Additionally, SNPs are biallelic (homozygous or heterozygous), generally stable to mutations, locus specific and co-dominant [31]. SNP information is easily available from generally expressed sequences tags (ESTs) on gene databases [15], with a low genotyping error rate compared to other markers [31], and therefore ideal for genetic studies and especially for assessing diversity [53]. For this study 93 SNPs were used (Table S2) to assess manioc diversity, tested previously by the team of the Manioc Genetics Laboratory of CIAT [78].

Samples were processed using the protocol for SNP genotyping with the EP1™ system and SNP type assays of Fluidigm® version S.01 following instructions from the manufacturer. The SNP assay is based on an allele-specific Polymerase Chain Reaction (PCR) SNP detection chemistry using a Biotium Fast Probe Master Mix, hybridizing SNPs at end-points and attaching signal bases that emit specific fluorescent patterns according to the DNA base that is read [79].

Prior to performing allele-specific PCR, a standard amount of manioc DNA (60 ng) per sample was amplified in a three-step procedure to obtain the sample assay. In step one a Specific Target Amplification (STA) with the manioc DNA was done in a thermocycler through 15 min at 95 °C for Taq polymerase activation, followed by 14 extension cycles of 95 °C for 15 s and 60 °C for 4 min. This is called the sample mix. In step two, the sample mix was diluted 1:100. In step three the diluted sample mix was combined with an assay mix which contained the ASP1, ASP2 primers and the locus-specific primer (LSP); the sample assay thus obtained was placed in the sample inlet of the chip of an IFC Controller HX. In the detector inlet a Fluidigm SNPtype™ custom assay based on a core set of 93 manioc SNPs was placed that was previously reported by Ferguson *et al.* [15] and validated at CIAT's Manioc Genetics Laboratory. After the sample assay and the SNPtype custom assay were placed in the chip, and the IFC Controller HX automatically set up reaction chambers. The 96.96 IFC was placed on the FC1™ cyclor and run using the following program: 95 °C 5 min then 4 cycles of 95 °C for 15 s, 64–61 °C for 45 s decreasing by 1 °C/cycle, then 72 °C for 15 s, followed by 34 cycles of 95 °C for 15 s, 60 °C for 45 s, 72 °C for 15 s, and finally 10 s at 25 °C.

SNP variability-data were captured in the EP1™ fluorescent reader at cycle 28 and 33 to be able to discriminate between homozygous and heterozygous SNP-allele calls. A direct detection of products was obtained as they emitted peaks at 495, 520, 538 and 554 nm that were read by a BioMark™ System for genetic analysis. Both data sets collected in the EP1™ (at 28 and 33 cycles) were analyzed using the Fluidigm SNP Genotyping Analysis software [80].

Sequences of samples collected in the field were compared with sequences from 99 samples of the CIAT's manioc world collection obtained with the same methodology. Samples of CIAT's manioc world collection included samples from South and Central America (71), Africa (1) and Asia (9), 3 hybrids and 15 landraces of unknown origin. Manioc DNA samples from the America's included: 5 from Argentina, 2 from Bolivia, 4 from Brazil, 26 from Colombia, 3 from Costa Rica, 3 from Cuba, 3 from Ecuador, 5 from Guatemala, 5 from Mexico, 2 from Panama, 2 from Paraguay, 5 from Peru, 1 from Puerto Rico, and 5 from Venezuela. The manioc DNA sample from Africa was from Nigeria. Manioc DNA samples from Asia included: 2 from China, 3 from Thailand, 2 from Indonesia, and 2 from Malaysia. The three samples of manioc hybrids were ICA-CIAT hybrids obtained by open or controlled pollination. The 15 samples from unknown origin were identified by the codes AM206-5, AM560-2, FLA 21, FLA61, FLA 19, GLA8, GM905-52, GM905-57, GM905-60, SM301-3, TMS60444, C18, SG107-35, GUT64, and JAC3. Results of manioc diversity of the CIAT core collection and samples of this study are presented as Figure S2 of our supplementary material.

5.6. Statistical Analysis

Chi-square tests were used to assess differences in manioc inventories and differences in the classes of manioc landraces cultivated among ethnic groups. The statistical analyses were performed with the Analytical Software Statistix 9.0.

A z-test was used to assess which morphological variables distinguished as different morphotypes plant pairs that were considered duplicates in the genomic analysis. The morphological description obtained for each variable was transformed into binary data (1 = when the morphological description of

the variable matched between genotype duplicates, and 0 = when the morphological description of the variable did not match between genotype duplicates). Z was then calculated as:

$$z = \frac{p - \pi}{\sqrt{\pi(1 - \pi)/n}} \quad (1)$$

where p was the number of times a variable chosen matched between genotype duplicates; π was the null hypothesis value that in this case was 0.5 as each variable has the same possibility to match or not between duplicates; and n was the sample size which corresponded to the number of genotype duplicates tested (21). The test considered that, when the values obtained for each variable were greater than the z -value from the table, the variable was significantly different among the 21 genotype duplicates compared. The variables that obtained the highest values were the variables that matched in most of the duplicates compared while variables with low scores could be potential morphologic discriminators of genotypes.

The sequences obtained from each manioc landrace through its genetic analysis were read, organized and analyzed to obtain the genetic structure of the population of the samples collected. The genetic structure was obtained using a Bayesian model approach [81] with the STRUCTURE 2.2 software [82]. This Bayesian analysis determines the minimum number of populations (K) that could have generated the observed diversity using an admixture model and assumes that each individual inherited some portion of its ancestry from each one of the K populations determined. The number of K populations is based on the rate of change in the log probability of the data between successive K values. To estimate it, a ΔK method [83] was implemented in the STRUCTURE software to ascertain the most likely value of K in this data set, using 1 to 10 populations. The length of the burn-in period was set on 100,000 and the number of MCMC Reps after burn-in on 200,000. After the value of K was determined, each sample was compared with each one of the K populations to discriminate samples into defined groups that segregate similarly. Information was used to elaborate Neighbor-Joining dendrograms of genetic diversity. The observed heterozygosity (H_o) of each SNP was calculated according to Nei [84] and Shete *et al.* [85] using PowerMaker software.

STRUCTURE outputs were processed using CLUMPP v1.1.2 [29] to account for the variability in individual membership probabilities across the different runs, and to find optimal alignments of independent replicates on each K . Neighbor-Joining dendrograms were elaborated with the data obtained with $K = 3$ and $K = 6$, calculated to be the number of populations that best explained the genetic data obtained [83]. Samples that, after being compared with three or six populations ($K = 3$ and $K = 6$) obtained membership coefficients (Q) higher than 0.9 for one of the populations with which they were compared, were considered samples with low genetic variability. On the contrary, samples that obtained membership coefficients (Q) between 0.9 and 0.3 in relation with all of the populations with which they were compared, were considered samples with high genetic variability; that is, the samples with the lowest values were the ones with the highest genetic variability.

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Author Contributions

The conceptualization of the research was done by Clara P. Peña-Venegas, Paul C. Struik, Gerard Verschoor, and Tjeerd Jan Stomph. Clara P. Peña-Venegas contributed collecting information in the field of manioc and social aspects, drafting the paper, doing the statistical analysis of the information, elaborating figures and tables, and preparing the final version of the manuscript. Tjeerd Jan Stomph contributed to the statistical analysis of the data, the elaboration of figures, writing the results and discussion, and in the final revision and editing of the paper. Gerard Verschoor contributed to collecting social information in the field and analyzing that information, writing the introduction and helping in the final editing of the paper. Luis A. Becerra Lopez-Lavalle contributed to the molecular analysis of the manioc samples, the analysis of the data, the elaboration of dendrograms and figures, and in writing the introduction and materials and methods. Paul C. Struik contributed to evaluating and structuring the information and the text, designing the tables and helping in the final editing of the text.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Olsen, K.M.; Schaal, B.A. Evidence on the origin of cassava: Phylogeography of *manihot esculenta*. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5586–5591.
2. El-Sharkawy, M.A. Cassava biology and physiology. *Plant Mol. Biol.* **2004**, *56*, 481–501.
3. El-Sharkawy, M.A. International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. *Photosynthetica* **2006**, *44*, 481–512.
4. Brown, A.H.D. Core collections: A practical approach to genetic resources management. *Genome* **1989**, *31*, 818–824.
5. Elias, M.; Panaud, O.; Robert, T. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers. *Heredity* **2000**, *85*, 219–230.
6. Elias, M.; McKey, D.; Panaud, O.; Anstett, M.C.; Robert, T. Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America): Perspectives for on-farm conservation of crop genetic resources. *Euphytica* **2001**, *120*, 143–157.
7. Elias, M.; Mühlen, G.S.; McKey, D.; Roa, A.C.; Tohme, J. Genetic diversity of traditional South American landraces of cassava (*Manihot esculenta* Crantz): An analysis using microsatellites. *Econ. Bot.* **2004**, *58*, 242–256.
8. Emperaire, L.; Peroni, N. Traditional management of agrobiodiversity in Brazil: A case study of manioc. *Hum. Ecol.* **2007**, *35*, 761–768.

9. Fraser, J.A. Caboclo horticulture and Amazonian dark earths along the middle Madeira River, Brazil. *Hum. Ecol.* **2010**, *38*, 651–662.
10. Boster, J.S. Exchange of varieties and information between Aguaruna manioc cultivators. *Am. Anthropol. New Ser.* **1986**, *88*, 428–436.
11. Salick, J.; Cellinese, N.; Knapp, S. Indigenous diversity of cassava: Generation, maintenance, use and loss among the Amuesha, Peruvian upper Amazon. *Econ. Bot.* **1997**, *51*, 6–19.
12. Zeven, A.C. Landraces: A review of definitions and classifications. *Euphytica* **1998**, *104*, 127–139.
13. Villa, T.C.C.; Maxted, N.; Scholten, M.; Ford-Lloyd, B. Defining and identifying crop landraces. *Plant Genet. Resour.* **2006**, *3*, 373–384.
14. Rabbi, I.Y.; Kulembeka, H.P.; Masumba, E.; Marri, P.R.; Ferguso, M. An est-derived SNP and SSR genetic linkage map of cassava (*Manihot esculenta* Crantz). *Theor. Appl. Genet.* **2012**, *125*, 329–342.
15. Ferguson, M.E.; Hearne, S.J.; Close, T.J.; Wanamaker, S.; Moskal, W.A.; Town, C.D.; de Young, J.; Marri, P.R.; Rabbi, I.Y.; de Villiers, E.P. Identification, validation and high-throughput genotyping of transcribed gene SNPs in cassava. *Theor. Appl. Genet.* **2012**, *124*, 685–695.
16. McKey, D.; Cavagnaro, T.R.; Cliff, J.; Gleadow, R. Chemical ecology in coupled human and natural systems: People, manioc, multitrophic interactions and global change. *Chemoecology* **2010**, *20*, 109–133.
17. Pujol, B.T.; Renoux, F.O.; Elias, M.; Rival, L.; McKey, D. The unappreciated ecology of landrace populations: Conservation consequences of soil seed banks in cassava. *Biol. Conserv.* **2007**, *136*, 541–551.
18. Alves-Pereira, A.; Peroni, N.; Abreu, A.G.; Gribel, R.; Clement, C.R. Genetic structure of traditional varieties of bitter manioc in three soils in Central Amazonia. *Genetica* **2011**, *139*, 1259–1271.
19. Fraser, J.A.; Alves-Pereira, A.; Junqueira, A.B.; Peroni, N.; Clement, C.R. Convergent adaptations: Bitter manioc cultivation systems in fertile anthropogenic dark earths and floodplain soils in central Amazonia. *PLoS One* **2012**, *7*, e43636.
20. Heckler, S.; Zent, S. Piaroa manioc varietals: Hyperdiversity or social currency? *Hum. Ecol.* **2008**, *36*, 679–697.
21. Duputié, A.; Massol, F.; David, P.; Haxaire, C.; McKey, D. Traditional amerindian cultivators combine directional and ideotypic selection for sustainable management of cassava genetic diversity. *J. Evol. Biol.* **2009**, *22*, 1317–1325.
22. Pujol, B.; Mühlen, G.; Garwood, N.; Horoszowski, Y.; Douzery, E.J.P.; McKey, D. Evolution under domestication: Contrasting functional morphology of seedlings in domesticated cassava and its closest wild relatives. *New Phytol.* **2005**, *166*, 305–318.
23. McKey, D.; Elias, M.; Pujol, B.; Duputié, A. The evolutionary ecology of clonally propagated domesticated plants. *New Phytol.* **2010**, *186*, 318–332.
24. Arias, J.C.; Aguila, L.R.D.; Huaines, F.; Acosta, L.E.; Camacho, H.A.; Marín, Z.Y. *Diversidad de Yucas Entre los Ticuna: Riqueza Cultural y Genética de un Producto Tradicional*; Instituto Amazónico de Investigaciones Científicas Sinchi: Bogotá, Colombia, 2004; p. 32. (In Spanish)
25. Acosta, L.E.; Mazorra, A. *Enterramientos de Masas de Yuca del Pueblo Ticuna: Tecnología Tradicional en la Várzea del Amazonas Colombiano*; Instituto Amazónico de Investigaciones Científicas, Sinchi: Leticia, Colombia, 2004; p. 109. (In Spanish)

26. Camacho, H. Màgutá. *La Gente Pescada por Yoi. Colcultura Premios Nacionales 1994*; Colcultura: Bogotá, Colombia, 1995. (In Spanish)
27. Peña-Venegas, C.P.; Stomph, T.J.; Verschoor, G.; Echeverri, J.A.; Struik, P.C. Classification and use of natural and anthropogenic soils by indigenous communities of the upper Amazon region of Colombia. *Hum. Ecol.* **2015**, in press.
28. Hammen, M.C.V.D. *La Dinámica de la Chagra*; Corporación Araracuara: Bogotá, Colombia, 1984. (In Spanish)
29. Jakobsson, M.; Rosenberg, N.A. Clumpp: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **2007**, *23*, 1801–1806.
30. Vieira, E.A.; Fialho, J.D.F.; Faleiro, F.G.; Bellon, G.; Fonseca, K.G.D.; Carvalho, L.J.C.B.; Silva, M.S.; Paula-Moraes, S.V.D.; Filho, M.O.S.D.S.; Silva, K.N.D. Divergência genética entre acessos açucarados e não açucarados de mandioca. *Pesqui. Agropecaria Bras.* **2008**, *43*, 1707–1715. (In Spanish)
31. Oliveira, E.J.D.; Ferreira, C.F.; Santos, V.D.S.; Jesus, O.N.D.; Oliveira, G.A.F.; Silva, M.S.D. Potential of SNP markers for the characterization of brazilian cassava germplasm. *Theor. Appl. Genet.* **2014**, *127*, 1423–1440.
32. Aristizábal, J.; Sánchez, T.; Lorío, D.M. *Guía Técnica Para Producción y Análisis de Almidón de Yuca*; FAO: Roma, Italy, 2007; Volume 163, pp. 2–3. (In Spanish)
33. Silva, J.T.D.; Paula, C.D.D.; Oliveira, T.M.D.; Pérez, O.A. Cassava derivatives and toxic components in Brazil. *Temas Agrar.* **2008**, *13*, 5–16.
34. Wilson, W.M.; Dufour, D.L. Why “bitter” cassava? Productivity of “bitter” and “sweet” cassava in a Tukanoan indian settlement in the northwest Amazon. *Econ. Bot.* **2002**, *56*, 49–57.
35. Chiwona-Karlton, L.; Mkumbira, J.; Saka, J.; Bovin, M.; Mahungu, N.M.; Rosling, H. The importance of being bitter: A qualitative study on cassava cultivar preference in Malawi. *Ecol. Food Nutr.* **1998**, *37*, 219–245.
36. Fresco, L.O. Cassava in shifting cultivation. In *A Systems Approach to Agricultural Technology Development in Africa*; Royal Tropical Institute: Amsterdam, The Netherlands, 1986.
37. Mühlen, G.S.; Alves-Pereira, A.; Clement, C.R.; Valle, T.L. Genetic diversity and differentiation of brazilian bitter and sweet manioc varieties (*Manihot esculenta* Crantz, Euphorbiaceae) based on SSR molecular markers. *Tipiti J. Soc. Anthropol. Lowl. S. Am.* **2013**, *11*, 66–73.
38. Arroyo-Kalin, M. The Amazonia formative: Crop domestication and anthropogenic soils. *Diversity* **2010**, *2*, 473–504.
39. Coomes, O.T. Of stakes, stems, and cuttings: The importance of local seed sustems in traditional Amazonian societies. *Prof. Geogr.* **2010**, *62*, 323–334.
40. Delêtre, M.; McKey, D.B.; Hodkinson, T.R. Marriage exchanges, seed exchanges, and the dynamics of manioc diversity. *Proc. Nat. Acad. Sci. USA* **2011**, *108*, 18249–18254.
41. Pautasso, M.; Aistaka, G.; Barnaud, A.; Caillon, S.; Clouvel, P.; Coomes, O.T.; Delêtre, M.; Demeulenaere, E.; Santis, P.D.; Döring, T.; et al. Seed exchange networks for agrobiodiversity conservation. A review. *Agron. Sustain. Dev.* **2013**, *33*, 151–175.
42. Samberg, L.H.; Shennan, C.; Zavaleta, E. Farmer seed exchange and crop diversity in a changing agricultural landscape in the southern highlands of Ethiopia. *Hum. Ecol.* **2013**, *41*, 477–485.

43. Dyer, G.A.; González, C.; Lopera, D.C. Informal “seed” systems and the management of gene flow in traditional agroecosystems: The case of cassava in Cauca, Colombia. *PLoS One* **2011**, *6*, e29067.
44. Wilson, W.M.; Dufour, D.L. Ethnobotanical evidence for cultivar selection among the Tukanoans: Manioc (*Manihot esculenta* Crantz) in the northwest Amazon. *Cult. Agric.* **2006**, *28*, 122–130.
45. Pereira, E. Palavra de coca e de tabaco como “conhecimento tradicional”: Cultura, política e desenvolvimento entre os uitoto-murui do rio caraparaná (co). *MANA* **2011**, *17*, 69–98. (In Spanish)
46. Fraser, J.A.; Clement, C.R. Dark earths and manioc cultivation in central amazonia: A window on pre-colombian agricultural systems? *Bol. Mus. Para. Emílio Goeldi* **2008**, *3*, 175–194.
47. Hastik, R.; Geitner, C.; Neuburger, M. Amazonian dark earths in bolivia? A soil study of anthropogenic ring ditches near baures (eastern llanos de mojos). *Erdkunde* **2013**, *67*, 137–149.
48. Arroyo-Kalin, M. Slash-burn-and-churn: Landscape history and crop cultivation in pre-columbian amazonia. *Quat. Int.* **2012**, *249*, 4–18.
49. Acosta, L.E. *Pueblos Indígenas de la Amazonia e Indicadores de Bienestar Humano en la Encrucijada de la Globalización: Estudio de Caso Amazonia Colombiana*; Universidad del País Vasco: Bilbao, Spain, 2013. (In Spanish)
50. Acosta, L.E.; Zoria, J. Ticuna traditional knowledge on chagra agriculture and innovative mechanisms for its protection. *Bol. Mus. Para. Emílio Goeldi* **2012**, *7*, 417–433.
51. Steward, A. Nobody farms here anymore: Livelihood diversification in the Amazonian community of carvão, a historical perspective. *Agric. Hum. Values* **2007**, *24*, 75–92.
52. Acosta, L.E.; Zoria, J. Experiencias locales en la protección de los conocimientos tradicionales indígenas en la Amazonia Colombiana. *Rev. Colomb. Amazon.* **2009**, *2*, 117–130. (In Spanish)
53. Kawuki, R.S.; Ferguson, M.; Labuschagne, M.; Herselman, L.; Kim, D.-J. Identification, characterisation and application of single nucleotide polymorphisms for diversity assessment in cassava (*Manihot esculenta* Crantz). *Mol. Breed.* **2009**, *23*, 669–684.
54. Ciarmiello, L.F.; Piccirillo, P.; Pontecorvo, G.; Luca, A.D.; Kafantaris, I.; Woodrow, P. A PCR based SNPs marker for specific characterization of English walnut (*Juglans regia* L.) cultivars. *Mol. Biol. Rep.* **2011**, *38*, 1237–1249.
55. Li, Y.-H.; Li, W.; Zhang, C.; Yang, L.; Chang, R.-Z.; Gaut, B.S.; Qiu, L.-J. Genetic diversity in domesticated soybean (*Glycine max*) and its wild progenitor (*Glycine soja*) for simple sequence repeat and single-nucleotide polymorphism loci. *New Phytol.* **2010**, *188*, 242–253.
56. Dulloo, M.E.; Hunter, D.; Borelli, T. *Ex situ* and *in situ* conservation of agricultural biodiversity: Major advances and research needs. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2010**, *38*, 123–135.
57. UNEP; ACTO. *Environment Outlook in Amazonia Geoamazonia*; The United Nations Environment Programme UNEP, The Amazon Cooperation Treaty Organization ACTO, The Research Center of the Universidad del Pacífico CIUP: Panama City, Panama, **2009**.
58. IGAC. *La Amazonia Colombiana y sus Recursos Proyecto Radargramético del Amazonas Proradam*; IGAC: Bogotá, Colombia, 1979. (In Spanish)
59. Duivenvoorden, J.F.; Lips, J.M. *Landscape Ecology of the Middle Caquetá Basin*; Tropenbos Colombia: Bogotá, Colombia, **1993**; Volume IIIA.
60. Shorr, N. Early utilization of flood-recession soils as a response to the intensification of fishing and upland agriculture: Resource-use dynamics in a large Tikuna community. *Hum. Ecol.* **2000**, *28*, 73–107.

61. Adams, C.; Murrieta, R.S.S.; Sanches, R.A. Agricultura e alimentação em populações ribeirinhas das várzeas do Amazonas: Novas perspectivas. *Ambiente Soc.* **2005**, *8*, 1–22. (In Portuguese)
62. Eden, M.J.; Andrade, A. Ecological aspects of swidden cultivation among the Andoke and Witoto Indians of the Colombian Amazon. *Hum. Ecol.* **1987**, *15*, 339–359.
63. USDA. *Soil Taxonomy a Basic System of Soil Classification for Making and Interpreting Soil Surveys*; USDA United States Department of Agriculture: Washington, DC, USA, 1999; Volume 436.
64. Piedade, M.T.F.; Worbes, M.; Junk, W.J. Geological controls on elemental fluxes in communities of higher plants in amazonian floodplains. In *The Biochemistry of the Amazon Basin*; McClain, M.E., Victoria, R.L., Richey, J.E., Eds.; Oxford University Press: New York, NY, USA, 2001; pp. 225–227.
65. Denevan, W.M. A bluff model of riverine settlement in prehistoric Amazonia. *Ann. Assoc. Am. Geogr.* **1996**, *86*, 654–681.
66. Glaser, B.; Birk, J.J. State of the scientific knowledge on properties and genesis of anthropogenic dark earths in central Amazonia (terra preta de índio). *Geochim. Cosmochim. Acta* **2012**, *82*, 39–51.
67. Glaser, B.; Haumaier, L.; Guggenberger, G.; Zech, W. The “terra preta” phenomenon: A model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **2001**, *88*, 37–41.
68. Lima, H.N.; Schaefer, C.E.R.; Mello, J.W.V.; Gilkes, R.J.; Ker, J.O.C. Pedogenesis and pre-colombian land use of “terra preta anthrosols” (“Indian Black Earth”) of western Amazonia. *Geoderma* **2002**, *110*, 1–17.
69. Glaser, B. Prehistorically modified soils of central Amazonia: A model for sustainable agriculture in the twenty-first century. *Philos. Transcr. R. Soc. B* **2007**, *362*, 187–196.
70. Acosta, L.E.; Mendoza, D. El conocimiento tradicional: Clave en la Construcción del Desarrollo sostenible en la Amazonia Colombiana. Available online: http://nuevo.portalces.org/sites/default/files/6_conocimiento_tradicional_clave_en_la_contruccin_del_desarrollo_sostenible_en_la_amazonia_colombiana.pdf (accessed on 9 December 2014).
71. Colombia, R.O. *Ley 99 de 1993*; Colombia, M.O.E.O., Ed.; Republic of Colombia: Bogotá, Colombia, 1993. (In Spanish)
72. Rosa, E.J.U.D.L. Los indios ticuna del alto Amazonas ante los procesos actuales de cambio cultural y globalización. *Rev. Esp. Antropol. Am.* **2000**, *30*, 291–336. (In Spanish)
73. Riaño, E. Los asentamientos ticuna de hoy en la rivera del río Amazonas colombiano. *Perspect. Geogr.* **2002**, *7*, 1–38. (In Spanish)
74. Andrade, A. *Investigación Antropológica de los Antrosols de Araracuara*; Fundación de Investigaciones Arqueológicas Nacionales: Bogotá, Colombia, 1986. (In Spanish)
75. CIAT. *Morphology of the Cassava Plant: Study Guide*; CIAT: Cali, Colombia, 1984.
76. Fukuda, W.M.G.; Guevara, C.L. *Descritores Morfológicos e Agronômicos para la Caracterização de Mandioca*; EMBRAPA/CNPMPF: Cruz das almas, BA, Brazil, **1998**; Volume 78.
77. Ministerio de Ambiente y Desarrollo Sostenible. Decreto por el cual se reglamenta el permiso de recolección de especímenes de especies silvestres de la diversidad biológica con fines de investigación científica no comercial. In *Decreto 1376 de 2013*; Ministerio de Ambiente y Desarrollo Sostenible, Ed.; Imprenta Ministerio de Medio Ambiente y Desarrollo Sostenible: Bogotá, Colombia, 2013; p. 12. Available online: <http://wsp.presidencia.gov.co/Normativa/Decretos/2013/Documents/JUNIO/27/DECRETO%201376%20DEL%2027%20DE%20JUNIO%20DE%202013.pdf>. (In Spanish)

78. Duitama, J.; Perea, C.S.; Ovalle, T.; Aranzales, E.; Ballen, C.; Calle, F.; Dufour, D.; Parsa, S.; Alzate, A.; Debouck, D.; *et al.* Revisiting cassava genetic diversity reveals eco-geographic signature of the crop's domestication. *Nat. Genet.* **2014**, submitted.
79. Li, J.; Deng, T.; Chu, X.; Yang, R.; Jiang, J.; Shen, G.; Yu, R. Rolling circle amplification combined with gold nanoparticle aggregates for highly sensitive identification of single-nucleotide polymorphisms. *Anal. Chem.* **2010**, *82*, 2811–2816.
80. Spurgeon, S.L.; Jones, R.C.; Ramakrishnan, R. High throughput gene expression measurement with real time PCR in a microfluidic dynamic array. *PLoS One* **2008**, *3*, e1662.
81. Kawuki, R.S.; Herselman, L.; Labuschagne, M.T.; Nzuki, I.; Ralimanana, I.; Bidiaka, M.; Kanyange, M.C.; Gashaka, G.; Masumba, E.; Mkamilo, G.; *et al.* Genetic diversity of cassava (*Manihot esculenta* Crantz) landraces and cultivars from southern, eastern and central Africa. *Plant Genet. Resour. Charact. Util.* **2013**, *11*, 170–181.
82. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959.
83. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620.
84. Nei, M. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA* **1973**, *70*, 3321–3323.
85. Shete, S.; Tiwari, H.; Elston, R.C. On estimating the heterozygosity and polymorphism information content value. *Theor. Popul. Biol.* **2000**, *57*, 265–271.