Biomass, Nutrient Content and Allometric Relations of Intensively Grown Cocoa (*Theobroma cacao*) Trees in Ecuador

MSc Thesis Plant Production Systems

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Contents

List of Abbreviations:

ha: Hectares

CV: Coefficient of Variation

PAR: Photosynthetically Active Radiation

SLA: Specific Leaf Area

CCN-51: Coleccion Castro Naranjal 51 cocoa variety

ANOVA: Analysis of Variance

SD: Standard Deviation

Log: Logarithm

LSD: Least Significant Difference

CEC: Cation Exchange Capacity

WUE: Water Use Efficiency

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Abstract

Theobroma cacao is an important cash crop in many tropical. Its production faces some major challenges, including a poor understanding of nutrient and biomass immobilisation and distribution. Furthering our knowledge in cocoa physiology could assist in closing yield gaps and reducing the need for forest clearance. The few available studies on nutrient and biomass distribution in *T. cacao* are old and originate from West-Africa. This research aimed to add fresh insights into these issues, bringing novelty in location and farming system sampled as the research was conducted on an intensive system in Ecuador.

Nutrient and biomass allocation and immobilisation in 1, 6, and 11-year-old *T. cacao* were investigated. Allometric models were established to allow non-destructive studies in the future. Foliar nutrient analyses were compared between leaves of the current flush, leaves of the previous and leaves in senescence to observe how *T. cacao* trees mobilise nutrients to support a new leaf flush. Correlations were analysed between N and Mg with SPAD values to allow fast, non-destructive foliar measurements in the future.

Tree biomass increased by significant amounts between 1, 6 and 11-year-old trees and the proportion of wood relative to leaves increased substantially with age. The average total biomass of a mature cocoa tree was 27.5 kg which equates to 31 tonnes of dry biomass per ha. Allometric equations were established to predict total biomass. *Total biomass = -0.392 + 0.11 × sum of trunk area at 30 cm* is used to predict total biomass with an R^2adj of 0.891. When this allometric equations was applied to predict biomass of 21-year-old trees on the same farm, a plateau was observed in biomass accumulation of mature cocoa trees. Total nutrient accumulation of mature *T. cacao* trees was observed in the following order: N > K > Ca > Mg > P > S > Mn > B > Fe > Cu > Zn. Contrary to expectations, the nutritional status of the trees appeared relatively low. Furthermore, no remobilisation was observed from old to young leaves and no macro or micro-nutrient differences were observed between tree components. No correlations were observed between SPAD units and either N or Mg.

This research shows some anomalies in what is known regarding biomass and nutrient distribution in *T. cacao.* More research is needed, particularly in studying mineral nutrition and partitioning so that these results can be better understood.

1. Introduction

Cocoa originates from the humid tropics of South America and is now a very important cash crop in tropical regions around the World (Bai et al., 2017; Motamayor et al., 2002). Its production is of particular importance for supporting the economies of many developing countries, and the livelihoods of the millions of smallholder farmers who dominate production in the global market (van Vliet and Giller, 2017). A better understanding of biomass production and nutrient allocation within biomass is important to improve management practises, better understand cocoa tree physiology and to aid in nutrient cycling studies (Dossa et al., 2008). Many aspects of cocoa physiology are poorly understood and not well documented which inhibits the development of effective farming practises. By measuring plant growth habits and nutrient dynamics on an intensive farm in Ecuador, the paper seeks to add depth to and challenge what is already known about important physiological traits in cocoa trees.

Cocoa is the main constituent of chocolate and recently has seen vast increases in production. The increase in demand is driven by new markets such as China and India (Squicciarini and Swinnen, 2016) and the trend towards consuming chocolate with a high cocoa content in Europe and America (Almeida and Valle, 2007). This has led to a world production increased of over 100% in the period between 1985 and 2014 (Wessel and Quist-Wessel, 2015). FAOSTAT estimated total world production in 2016 to be 4,5 million tons, and despite radical expansion in production, the world market is predicted to be entering a supply deficit in the coming years (Almeida and Valle, 2007; Laliberté et al., 2012). Increase in cocoa production has almost entirely been through increase in area (Oberthür et al., 2018). Plantations tend to expand into natural forests so the sector has been a main driver of deforestation (Ruf et al., 2015). With further expansions looming, the threat to forests in these areas is ever present (Gockowski and Sonwa, 2011). Despite the importance of the industry, both yield and knowledge gaps are large. Furthermore, relatively little is known about nutrient dynamics and biomass partitioning in cocoa trees (van Vliet and Giller, 2017), and a deeper understanding of this could help to bridge these gaps.

Sustainable intensification is a good alternative to meet the markets requirements whilst putting less pressure on natural forests by increasing production on current land. Sustainable intensification seeks to increase output and therefore reduce the yield gap, whilst minimising environmental impact (Rockström et al., 2017). A major step towards realising sustainable intensification in the cocoa industry will be to drastically improve the current fertilisation practices and to apply more modern farming techniques on small-scale plantations.

1.1. Production Systems

Approximately 70% of cocoa production comes from West African countries, particularly Côte d'Ivoire and Ghana who are the largest producers in the world (Aikpokpodion, 2010; Wessel and Quist-Wessel, 2015). The remainder of the world's cocoa is sourced from Latin America (Ecuador, Brazil and Columbia) and Asia (Indonesia, Malaysia and Papua New Guinea) (WCF, 2014). The production norms vary slightly between countries and regions but he majority of farms occupy less than 5 hectares (ha) of land (Somarriba et al., 2014). They entail a low input system with pioneer cropping into forest margins which take advantage of residual forest fertility and shade (Gockowski et al., 2013; Ruf et al., 2015; Wessel and Quist-Wessel, 2015). Replanting in West Africa is not common practice, with most farmers migrating into forest frontiers once their farm becomes unproductive (Ruf et al., 2015). Extensive systems such as this suffer from substantial yield gaps. For instance, Aneani and Ofori-Frimpong (2013) discovered that cocoa yields in Ghana vary from as low as 60 kg/ha to 2000 kg/ha where the national average yield is 340 kg/ha. In the same study, an experimental station averaged yields of 1900 kg/ha over four years, leading to an experimental yield gap of 1 553 kg/ha. Yields of 6300 kg/ha have been achieved on an experimental site in Malaysia (Yapp and Hadley, 1991) showing the stark differences in what farmers can achieve. Similarly, maximum yields of 6100 kg/ha were found to be achievable by a model based on Malaysia's growth conditions (Zuidema and Leffelaar, 2002). The same model predicted achievable yields of 5000 kg/ha in Ghana.

These yield gaps arise because farmers fail to optimise limiting factors (water and nutrients) and reducing factors (weeds, pests, diseases and pollutants). With respect to cocoa production, major restrictions to yield come from using old varieties, pests and diseases, poor nutrient management in the soil and a lack of pollinators (van Vliet and Giller, 2017). This is related to the fact that many farmers currently use poor management practices and fail to adopt new farming technologies (Aneani and Ofori-Frimpong, 2013). Maintaining optimum nutrient availability to trees is important to close the yield gap in cocoa production because plantations gradually deplete the soil of nutrients (Gockowski and Sonwa, 2011; Wessel, 1971). Fertilization practices are surprisingly uncommon in extensive cocoa production systems due to a lack of capital (van Vliet and Giller, 2017), lack of accessibility to fertilisers (Aneani and Ofori-Frimpong, 2013) and lack of willingness to invest in fertilisers because of the risk of making a loss (Oberthür et al., 2018). Farmers see the investment as being too risky to justify the initial investment because i) fertilisers are expensive, ii) the benefits are often not seen due to the vagueness and inaccuracy of fertiliser recommendations, iii) the risk of yield loss to other uncontrollable factors such as weather and pests and diseases, and iv) very low cocoa prices.

Current fertiliser recommendations are unreliable, highly inconsistent, and many do not consider spatial or seasonal differences in nutrient requirements. For example, according to Snoeck et al. (2010), the current blanket fertiliser recommendation in Ghana is only suitable for 6% of the production area. Many recommendations do not reflect that the trees nutrient requirements will vary with age or development stage (Thong and Ng, 1980). According to Oberthür et al. (2018), most of the current knowledge involving cocoa was developed in West Africa and Malaysia. As a result, major production areas such as Indonesia and Ecuador lack any meaningful fertilizer recommendations specific to their conditions. Any trials that have been done should only be applicable to the specific soil, climate, variety, etc of the location where the trial was carried out (Snoeck et al., 2010).

Besides thinned forest shaded systems which are extensive, Gockowski et al. (2013) identified a further two important cocoa production systems: i), intensive full sun (high technology) systems and ii) certified shade grown systems which receive a price premium for sustainably produced cocoa. Full sun (high technology) systems will be the focus of this study. Intensive cocoa farms tend to be better managed. They use better varieties, fertilise more thoroughly, spray more frequently for pest and disease and prune their trees meticulously. Mechanisation is also common in pruning, establishing drainage, and processing of beans (particularly drying).

Ecuador offers a very diverse landscape with local environmental conditions varying substantially. As a result, the approach to growing cocoa depends on the region. The country is renowned for its high-quality cocoa beans from 'Nacional' variety trees which fetch a market premium for their 'fine flavour.' Typical cocoa farms in Ecuador have low productivity due to low-yielding planting material, old trees, and susceptibility to diseases such as witches' broom disease (Amores et al., 2011). As a result, many farmers have replaced the Nacional varieties with the clonal variety CCN-51. This variety is more resilient against sun damage and disease and is high yielding. It is because of these properties that the variety can be grown more intensively, without shade and with heavy pruning to stimulate fruit production. The farm where this study was carried out is intensive, taking advantage of these properties. As a result, the system in this study varies substantially from a typical cocoa farm in Ecuador. Some major differences are that trees are hedged and pruned on a regular basis and chemicals are applied to control pests and diseases. Fertilisation is done through the irrigation system on a bi-monthly basis and the system is a monoculture with trees sown at a relatively high density.

Understanding the effects of intensified cocoa practices on tree growth is important to advance our knowledge of cocoa tree physiology under different growing conditions. This will help in understanding the factors which play a role in yield production (Bastide et al., 2009). Recognising the nutrient dynamics of intensive cocoa systems will aid in nutrient cycling studies and comparisons

because nutrient management is one of the major areas of difference between intensive and extensive systems (Dossa et al., 2008).

1.2. Biomass Allocation and Immobilisation

Cocoa trees are known to exhibit rapid vegetative growth in the first 3 to 4 years as the tree establishes itself, after which biomass increase is more static and will often plateau at a certain point. A recent study by Calvo Romero (2018) shows a sharp increase in total tree biomass between 1.5 and 5-year trees to approximately 55 kg per tree. This was followed by an evident plateau in growth shown by the biomass of 10 and 19 year-trees (**Fig. 1**). A similar trend was shown by Thong and Ng (1980) in **Fig. 2** except with a slightly lower and more variable total biomass.

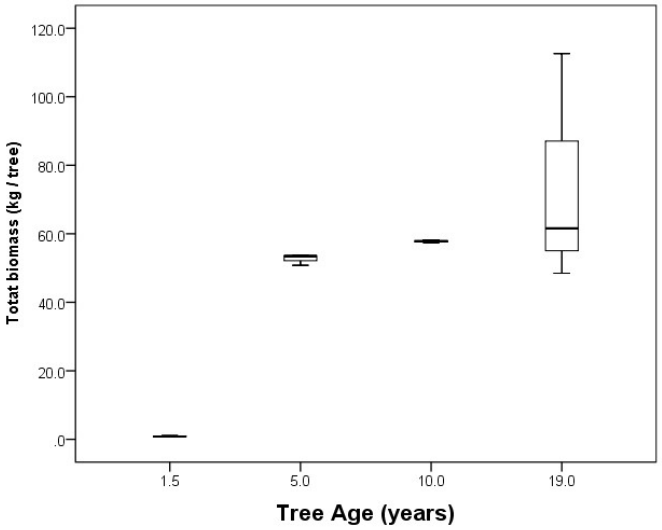

Figure 1: Total T. cacao biomass per tree at 1.5, 5, 10 and 19 years old (Calvo Romero, 2018)

Figure 2: Progression of total dry matter per plant with plant age of *T. cacao(Thong and Ng, 1980)*

Besides age, several factors have an influence on total biomass of cocoa trees including planting density, level of shading, local environmental conditions (including radiation levels, nutrient availability and temperature), and cultivar (Alpizar et al., 1986; Hartemink, 2005). This is shown in **Table 1** as biomass varies considerably between each of the studies. This makes the available research into biomass allocation and immobilisation highly inconsistent. Many authors did not include the roots in their measurements and did not classify the farming system clearly. Isaac et al. (2007) used allometric equations which were not developed specifically for cocoa in their comparison between total biomass in an agroforestry and monoculture system. Furthermore, no such studies are available for an intensive system.

Table 1: Tree biomass of *T. Cacao* (whole tree or above-ground fraction) of various studies done on a range of tree ages, tree densities, locations and farming systems.

Tree Age	Tree Density (trees/ha)	Components	Biomass (t/ha)	Location	Farming System	Source
1.5	1333	Whole Tree	0.6*	Ivory Coast	Agroforestry	(Calvo Romero, 2018)
5	1333	Whole Tree	77.3*	Ivory Coast	Monoculture	
10	1333	Whole Tree	80*	Ivory Coast	Monoculture	
19	1333	Whole Tree	85*	Ivory Coast	Agroforestry	
5	-	Whole tree	36	Malaysia	30% shade	(Thong and Ng, 1980)
10	-	Above-ground	8.4-9.8	Costa Rica	Moderate Shade	(Alpizar et al., 1986)
30	1000	Trunk and Branches	10	Cameroon	Moderate Shade	(Boyer, 1973)
30	1000	Trunk and Branches	15	Cameroon	Full sun	
30	997	Whole Tree	17.1	Venezuela	Agroforestry	(Aranguren et al., 1982)
8	1100	Above-ground	22.8	Ghana	Monoculture	(Isaac et al., 2007)
8	1100	Above-ground	41	Ghana	Agroforestry	
5	625	Whole tree	10.7	Indonesia	-	(Hartati and Prastowo, 2017)
30	625	Whole tree	14	Indonesia	-	

-: unknown, *: use data with caution

Figure 3 shows the temporal changes in component biomass allocation in cocoa trees and **Table 2** shows the allocation of mature cocoa trees. With time, less biomass is partitioned to leaves and relatively more to the woody biomass of the tree. There is substantial variation in the values in **Table 2** which may be because in the study by Bastide et al. (2009), there is no clear indication of the range in tree ages as the trees sampled were selected based on their average production. The two studies

also differ in farming system with the study by Thong and Ng (1980) being done in shaded trees and in the study by Bastide et al. (2009), the trees were exposed to full sun. Further studies in this field have been done but the results are hard to interpret so cannot be used for comparison of biomass distribution.

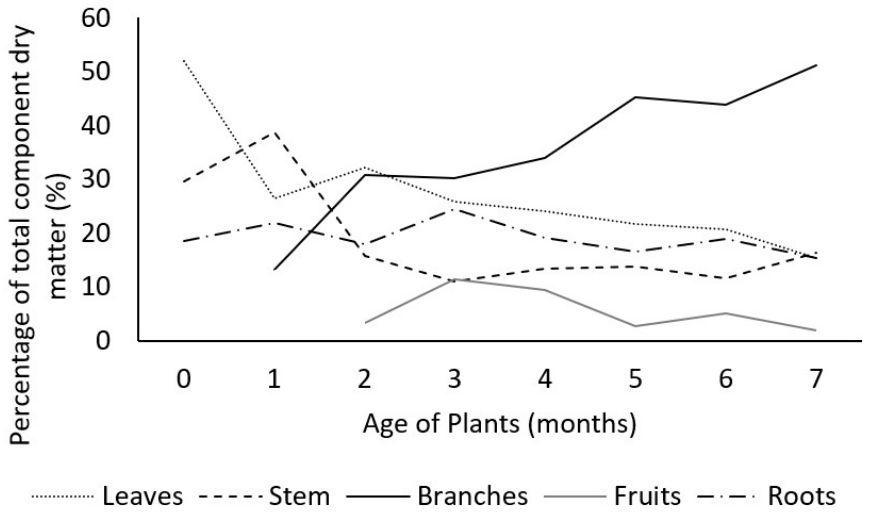

Figure 3: Component biomass allocation as a percentage of total dry biomass of *T. cacao* at different development stages (Thong and Ng, 1980)

Table 2: Distribution of biomass to various tree components for mature trees (>5 years) according to Bastide et al. (2009)

Leaves	Shoots	Branches	Trunk	Taproot	Roots
9.7	19.0	31.6	19.2	10.8	9.7
CV=29%	CV=43%	CV=25%	CV=20%	CV=22%	CV=24%
	Aerial parts 79%			Underground parts 21%	

CV: Coefficient of variation

Besides allocation between tree components, it is also interesting to look at allocation of resources at different tree heights. It is well documented that leaves acclimatise to photosynthetically active radiation (PAR) levels to maximise their photosynthetic capabilities (Kitao et al., 2018; Lambers et al., 2008; Oikawa et al., 2005; Pearcy and Sims, 1994). Lower canopy plants or shade leaves must maximise their light capture whilst minimising C loss and energy usage. Leaves exposed to high light intensities on the other hand must make efficient use of the available energy whilst mitigating the risk of light induced damage (predominantly by photoinhibition) (Pearcy and Sims, 1994). This is particularly

important in *T. cacao* as they are a shade adapted species. Leaves that develop in a relatively shady environment tend to invest more resources in their leaf area to maximise their chances of coming in to contact with a sun speck. This goes hand in hand with a lower Specific Leaf Area (SLA) as these leaves are thinner and have a lower density of mesophyll cells which allows for more light scattering within the leaf (Lambers et al., 2008).

1.3. Allometric Models

Allometric models are mathematical relationships which predict total or component biomass non-destructively, using measurable parameters such as trunk diameter or tree height (Andrade et al., 2016; Parresol, 1999; Picard et al., 2012). These models are based on the assumption that under uniform growing conditions, trees of the same variety, irrespective of tree size will follow the same rules of growth (Dietze et al., 2008; King, 1996; Weiner, 2004). Allometric models are commonly used in forestry to estimate a tree's size and biomass but they are also useful in perennial plantations such as cocoa in understanding growth patterns, and in carbon sequestration studies. Seeing as cocoa is so strongly associated with deforestation and land use change, it is important to understand biomass and carbon stocks of various cocoa systems and natural systems to get insights into the impacts of such land-use changes. Allocation of biomass to different components also follows a predictable growth trajectory, so analysing partitioning through allometric models should also be possible (Weiner, 2004).

Few such models are available for cocoa plantations, and none were found that were established on an intensively farmed system (**Table 3**). Furthermore, these existing studies did not construct component allometric equations.

Table 3: Existing allometric equations of different locations and farming systems used to estimate total or above-ground biomass of cocoa, coffee and dry tropical forests

Model	Location	Farming System	Trees Sampled	Source
$LogB = (-1.684 + 2.158 \times Log(D30) + 0.892 \times Log(H))$	Costa Rica	Cocoa-Unknown	Unknown	CATIE (unpublished)
$AGB = 0.202 \times D30^{2.112}$ $Root\ B = 0.142 \times D30^{2.269}$	Indonesia	Cocoa-Agroforestry	90	(Smiley and Kroschel, 2008)
$B = 1.9114 \times DBH^{1.1259}$	Indonesia	Cocoa-Agroforestry	35	(Toknok, 2011)
$Log_{10}AGB = -1.181 + 1.991(log_{10}DBH)$	Nicaragua	Coffee(pruned)-Agroforestry	92	(Segura et al., 2006)
$AGB = -0.357 + 0.371 \times D15$	Nicaragua	Coffee(pruned)-Agroforestry	92	(Segura et al., 2006)
$AGB = 0.2035 \times DBH^{2.3196}$	General	Dry tropical forest	504	(Pearson et al., 2005)

B: Total Biomass, AGB: Above-ground biomass, D30: Trunk diameter at 30 cm, D15: Trunk diameter at 15cm, H: height, DBH: Trunk diameter at breast height

1.4. Nutrient Accumulation in Cocoa Trees

Allocation of nutrients to various tree components varies with tree development stage. For example, Thong and Ng (1980) found significant variations in nutrient distributions when comparing nursery cocoa (5-12 months), immature cocoa (12-28 months) and mature cocoa. They found that in nursery cocoa, the leaves played the most important role in nutrient storage of all nutrients. For immature cocoa, the distribution is similar but for mature cocoa, both the leaf and the woody component gathered nutrients in similar quantities. This highly related to biomass capture so whether the component nutrient concentrations changes are unclear from this study. **Table 4** shows the expected total nutrient content per ha of cocoa plants at different ages. Once again, rapid increase in nutrient capture is seen in the initial 3-4 years followed by a plateau. K is captured in the highest quantity in this study followed by N and Ca. This study agrees strongly with the study by Ling (1984) (**Fig. 4**). However, Alpizar et al. (1986) had contrasting results showing that nutrients were captured in the following order: N>Ca>K>Mg>P. The reason for this is unclear, however it could be because Alpizar et al. (1986) never considered the nutrient content of fruits and flowers which have a relatively high K concentration (Thong and Ng, 1980; Wessel, 1985).

Table 4: Nutrient accumulation of cocoa plants (total biomass and fruits)(kg/ha) at different ages(Thong and Ng, 1980)

Age of plants (months)	Nutrient Composition of cocoa plants (kg/ha)						
	N	P	K	Ca	Mg	Mn	Zn
5	0.4	0.1	0.5	0.3	0.1	0.005	0.002
12	4.3	1.1	4.3	4.3	2.1	0.08	0.02
28	136	14	151	113	47	3.9	0.5
39	212	23	321	140	71	7.1	0.9
50	387	31	420	170	90	5.7	1.1
61	399	44	577	331	129	4.8	1.4
72	407	54	551	378	126	5.4	1.7
87	559	53	669	482	152	5.6	2.1

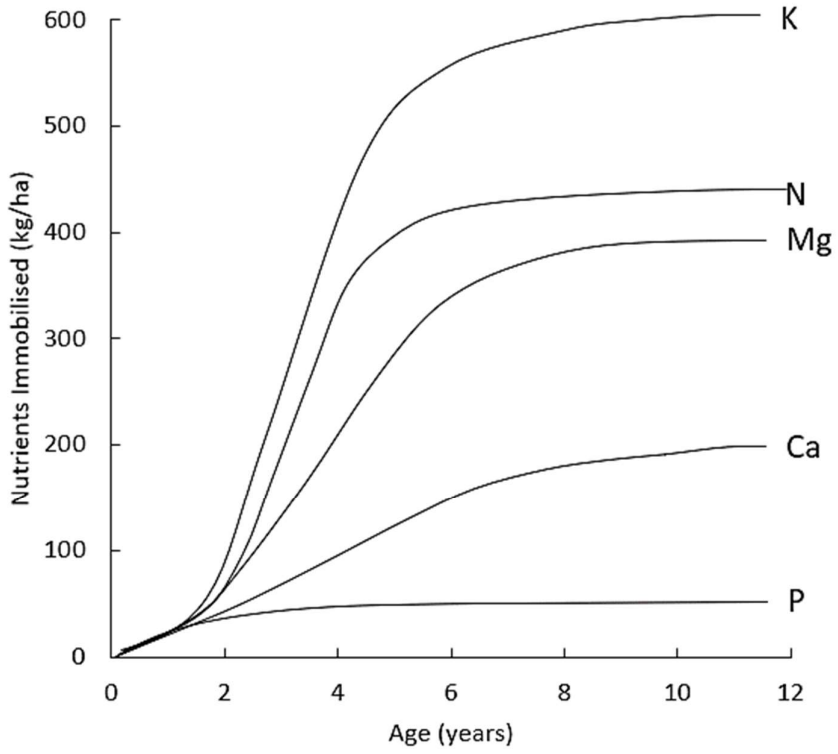

Figure 4: Nutrient accumulation in cocoa (Ling, 1984)

T. cacao leaves go through a natural flush cycle with periods of growth and quiescence, replacing almost all the leaves yearly (Almeida and Valle, 2007; Hutcheon, 1976). Under nutrient limiting conditions, plants often drop older and/or lower canopy leaves and reallocate the nutrients and photoassimilates to younger leaves (Kikuzawa and Lechowicz, 2011). Such is the case with cocoa leaf nutrient dynamics as a new flush often coincides with senescence of older leaves and a marked drop in N content of mature cocoa leaves (Almeida and Valle, 2007; Santana and Igue, 1979).

Hand-held leaf chlorophyll meters (SPAD meters) such as the MC-100 chlorophyll concentration meter take leaf chlorophyll measurements rapidly, cheaply and non-destructively. This is vastly more practical than the traditional methods which involved extraction of leaf materials and spectrophotometric determination. Such devices are often used to measure leaf N and Mg because much of these elements are constituents of leaf chlorophyll (Costa et al., 2001; Netto et al., 2005; Ruiz-Espinoza et al., 2010). These hand-held meters measure the ratio of transmittance of red light which is strongly absorbed by chlorophyll, and near infrared light which is not absorbed by chlorophyll. The relationship between SPAD units and either chlorophyll or nutrients is nonlinear and species dependent (Markwell et al., 1995), hence the need for calibration curves to be established. To date, only one attempt has been made to explore the relationship between SPAD units and leaf N or Mg concentration in *T. cacao* by Calvo Romero (2018) who found no significant correlations. If a calibration curve can be established, it will be very useful for agronomic and scientific purposes. It would help with giving an indication of the nutritional status of the plant, showing nutrient uptake efficiency or rate, understanding nutrient allocation etc.

2. Research Questions and Hypothesis

As already mentioned, there is a shortage of research in cocoa nutrition. Furthermore, nutrient and biomass data is unavailable for intensively farmed cocoa systems. Much of the available research is old and due to changing circumstances, needs to be updated. This research aims to add new insight into the topic of nutrient and biomass distribution, with the hope that it can be used in to aid in nutrient cycling studies, designing fertiliser recommendations and better understanding *T. cacao* physiology.

The research questions of the study are listed below with the hypothesis that have been made following an in-depth literature analysis:

Research Question 1: How does tree age relate to a) total tree biomass (per tree and per ha) and b) biomass allocation?

Sub Question 1.1: Does canopy position play a role in allocation of assimilates?

Hypothesis 1a: *Per tree the biomass in Ecuador is less than what has been observed in West Africa because of greater competition between trees and more thorough pruning.*

Hypothesis 1b: *The biomass allocation will vary with age, particularly that the proportion of wood will increase with age – leading to a smaller proportion of leaves. The largest difference will be between the 1 year old and 6-year-old trees.*

Hypothesis 1.1 *Canopy position and henceforth light exposure will affect leaf area and new leaf production. With increasing canopy height (and therefor light exposure), leaf area will decrease, and current flush leaf production will increase.*

Research Question 2: Can allometric models accurately predict biomass accumulation?

Hypothesis 2: *Making allometric models will be possible, with an accuracy of at least 75% (ideally should be higher but with the limited sample it is not likely).*

Research Question 3: What is the total nutrient immobilisation in an intensive cocoa field at different ages and what is the order of importance of macro and micro-nutrients in the system? What is the importance of the various tree components in terms of macro and micro-nutrient allocation?

Sub Question 3.1: How effective is the mobilisation of nutrients from old to young leaves?

Sub Question 3.2: Can a SPAD calibration curve be established for N and Mg?

Hypothesis 3*: Immobilisation of nutrients will be in greater amounts than what was seen by Ling (1984) and Thong and Ng (1980)(see section 1.2) because nutrients are supplied in luxurious quantities. The order of importance of nutrients will be the same as well. Vegetative (leaves) and reproductive tissue (fruit) should be more nutrient rich than the storage organs (wood and roots). Husks will have high K concentrations.*

Hypothesis 3.1 *Current flush leaves will mobilise much of the nutrients from older leaves. Therefore, old leaves (particularly from leaves in senescence) will be depleted of all nutrients except Ca and B as they are immobile.*

Hypothesis 3.2 *Establishing a SPAD calibration curve with N and Mg will be unsuccessful because nutrients are very dynamic in and between cocoa leaves*

3. Methods

3.1. Study Area

The study ran from 26 February 2018 until 20 April 2018 on La Chola Farm in the Guayas Province of Ecuador, situated 60 km South-West of Guayaquil (**Fig. 5**). The region has a tropical savanna climate with a pronounced dry season between June and November where rainfall is typically lower than 60mm per month. Most of the expected annual rainfall of roughly 1250 mm falls between January and April when the climate is hot and humid. In these months, heat builds up during the day and heavy showers in the late afternoon are common. Average daily temperatures range from 25°C in July to 28°C in March.

La Chola is a high yielding cocoa farm, producing primarily for the export market. Unlike most cocoa plantations in the world, La Chola is a well-managed, irrigated commercial farm, with high levels of mechanisation and fertiliser applications on a bi-monthly basis. The trees are hedged and pruned regularly, and chemicals are applied to control pests, diseases and weeds. Soils are typically inceptisols, with textures ranging from loam to silty clay-loam. Generally, the soils are fertile but have a low organic matter content.

Figure 5: Farm Location

3.2. Experimental Design

The experiment was conducted on *Theobroma cacao* clones of the Coleccion Castro Naranjal (CCN 51) variety, which is a high yielding variety common in Ecuador. The experimental design is illustrated in **Table 5**. Three tree ages were tested which are labelled as ages 1, 6 and 11 for simplicity. However,

the exact ages are as follows: the 6-year trees were all planted between January and August 2012 depending on the block. The 11-year block was established in October 2006. The exact planting date for the 1-year trees is unknown. All trees were harvested in a random order between 12/03/2018 and 12/04/2018.

Figure 6: Representative trees from each age; 1, 6 and 11 from left to right

For each age, as wide an area as possible was sampled. That way, local soil differences would counteract each other. Eight to ten sub-plots per tree age were established by dividing the area using google earth. A sub-plot was randomly selected, then a random tree within the sub-plot was selected. This was done by eliminating border trees from the selection and randomly selecting a column and row from the sub-plot. If the tree was healthy, with no signs of pests and diseases then it was used. In the rare occasion that the tree never met the criteria, another random column and row was selected within the same sub-plot. Once a sub-plot was used, it was eliminated from further selections. The GPS location of all the sampled trees was taken and superimposed over the extensive soil map of the farm using Google Earth (**Fig. 7**). The one-year-old trees are very close together because their block represented a replanted area within a block of older trees.

Table 5: Sampling design and descriptive information of lots

Tree Age	1	6	11
Variety	CCN51	CCN51	CCN51
Sampled trees	5	5	5
Plant Density (trees/ha)	1500	1500	1130
Row type	Single row	Double row	Single row

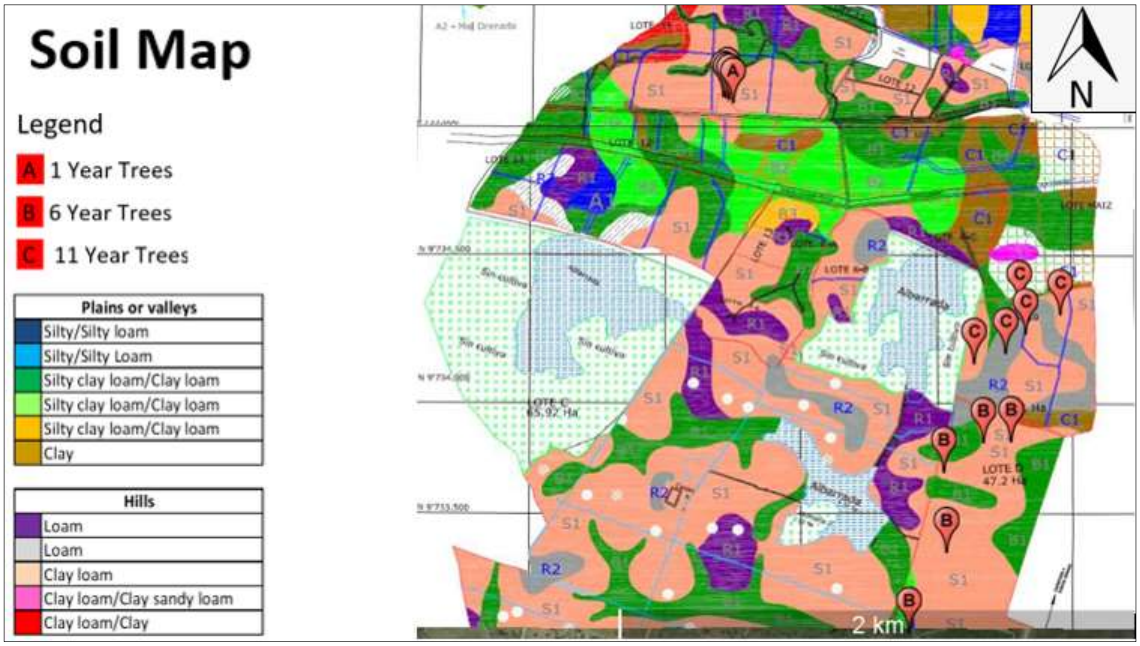

Figure 7: Site map including soil types

3.3. Determining Total Biomass and Biomass Distribution

Determining above and below-ground tree biomass and distribution was done by destructively harvesting and separating the entire tree into its components. These were the shoots (branches less than 1 cm in diameter and containing an apical meristem), branches, leaves (upper middle and lower canopy)), fruit (pods and husks), lateral roots and tap root. A summary of the samples taken per tree is provided in **Table 6**. One-year-old trees yielded slightly less samples because they were not yet bearing fruit and their canopy was too small to be divided into 3 sections. As a result, their canopies were divided in two; top and bottom.

Table 6: Samples taken per tree

Leaves	Top current flush (TLCF)
	Top previous flush (TLPF)
	Middle current flush (MLCF)
	Middle previous flush (MLPF)
	Bottom current flush (BLCF)
	Bottom previous flush (BLPF)
	Whole canopy leaves in senescence (LS)
Branches	(Shoots <1cm in diameter)
	(Branches >1cm in diameter)
Trunk	
Roots	Tap
	Laterals
Fruit	Beans
	Husks

3.3.1. Above-Ground Biomass

The canopy was visually divided into three sections; the bottom quarter, the middle half, and the top quarter. The leaves were collected and piled according to the canopy position upon which they sprout. At the same time, leaves of the current flush were separated from leaves of previous flushes for each canopy position and a further separation was made consisting of leaves in senescence for the entire canopy. Current flush leaves were distinguishable by their colour (initially red due to the presence of anthocyanins and then progressing to light green), their proximity to the apex of shoots, the angle of the petiole relative to the shoot (LCF usually tilted downwards) and the petiole of the leaves was narrower and made of softer tissue than older leaves (see **Appendix B**). Leaves in senescence were easily identifiable as they consisted of yellow or grey, mostly dead tissue. Felling and separation of the trees and their components was done using a handsaw, pruning shears and a machete. Once all branches were removed, the trunk was separated from the tap root as close to the soil surface as

possible. The fresh weight of each component was weighed in the field using a 0-100 kg field scale or a 0-5kg kitchen scale (for smaller samples) and the weight of the jute sack or plastic bag which contained the component was subtracted from the total. A well homogenised subsample of about 100-500 g fresh weight for woody tissue and 50 g for leaf tissue was taken and stored in a zip lock. Homogenisation of the samples aimed to make the subsample representative of the total mass. For example, subsamples of lateral roots were made up of fine and woody root tissues in proportion to their respective contribution to the overall lateral root biomass. The subsamples were stored in a cooler box in the field and refrigerator thereafter whilst awaiting transportation to the laboratory. Special care was taken to weigh leaves and create subsamples quickly so that they would not perish in the heat. The subsamples were weighed before and after drying them at 70 ^{o}C until dry (when the mass was constant). The dry biomass of each component was estimated gravimetrically using the fresh to dry weight ratio of the subsample multiplied by the total fresh weight of that component. The sum of the weight of all components gives the total biomass of a tree. This value will be extrapolated to the total biomass per ha by multiplying the average biomass at each age by the tree density per ha.

Figure 8: **Left***:* Using the petiole leaf area app on a cell phone mounted on a tripod to measure leaf area, **centre***:* Shoots from separate areas of the canopy, homogenised for a subsample, **right***:* Weighing branches in the field

Figure 9: top left: a leaf sample on the canvas prior to weighing and taking a subsample, **top centre:** removing the tap root and base from the soil, **top right:** making subsamples using a chainsaw, **bottom Left**: Exposing the tap-root (up to 1.5 m deep), **bottom centre:** leaf sub-samples ready to go to the laboratory, **bottom right**: washing lateral roots

3.3.2. Root Biomass

Like above, the root components (tap root and lateral roots) were separated, and their biomass was quantified by determining the fresh to dry weight ratio of the samples. To expose the roots, a perimeter was established around the tree of interest using a rope and pegs. The size of the perimeter depended on the distance to neighbouring trees (the rope was placed exactly between the tree of interest and the neighbouring trees on all four sides). Within the perimeter all roots were removed up to 30cm deep as cocoa is described as a surface root feeder (Aikpokpodion, 2010; Thong and Ng, 1980). The tap-root was dug until the bottom was found. This was normally at 1 to 1.5 m deep for mature trees. An assumption was made that overlapping of lateral roots between the sampled tree

and neighbouring trees will balance each other out at the perimeter. Both the tap and lateral roots were washed thoroughly to remove the soil before weighing or taking subsamples.

3.3.3. Canopy Position Analysis

Leaf area measurements were taken on LPF for each canopy position using the Petiole leaf area app. Per tree, 10 leaves were measured for each canopy position. The leaves were randomly selected from the subsamples taken in 3.3.1. To observe allocation of new leaf growth, dry mass for LCF at each canopy position (see 3.3.1.) was taken as a percentage of the total LCF biomass per tree.

3.4. Determining Total Nutrient Content and Nutrient Allocation

3.4.1. Destructive Nutrient Determination

Leaf and woody tissue were dried at 70 °C in an oven and ground to a powder using a mill. Extraction of nutrients was done by wet digestion for N, P, K, Ca and Mg, and by Calcination for Fe, Mn, Cu and Zn. Quantification was done by; Atomic Absorption Spectrophotometry using a Perkin Elmer AAnalist 400 for K, Ca, Mg, Fe, Zn, Cu and Mn (Piccolo and O'Connor, 1968), the Macro Kjedahl method for N as described by Bremner (1996), and visible UV were observed spectrophotometrically using a JASCO V630 for P, S and B (Islam et al., 2003). WEPAL reference samples were analysed concurrently to verify the accuracy of the nutrient samples.

Once the component nutrient concentration is known per unit biomass, it can be multiplied by the total biomass of that component to quantify total nutrient immobilisation per component and per tree. This can once again be extrapolated to determine the total amount of nutrients immobilised per ha for each age of *T. cacao* tested.

3.4.2. Establishing a SPAD Calibration Curve

Leaf measurements were taken with a SPAD meter (MC-100 chlorophyll concentration meter) to determine leaf chlorophyll content which is proportional to the N and Mg content of the leaf(Lin et al., 2010). For each canopy position, 10 leaves of each of the categories, LCF and LPF and overall LS were randomly selected from the subsamples obtained in 3.3.1. For each leaf, 2 readings were taken on either side of the central rib on the adaxial surface. A scatter-plot was made between SPAD values and leaf nutrient concentrations of N and Mg to observe any correlations.

3.5. Establishing Allometric Models

3.5.1. Variables

The dependent variables analysed in this study were average biomass per tree for each major component (roots, leaves and wood component) and total biomass. The independent variables were

tree height, canopy area and the sum of the cross-sectional area of the trunk or branches at 30 cm above ground.

Before the trees were destructively harvested (3.3.1 and 3.3.2), dimensions for basal trunk diameter at thirty cm above ground, tree height, and canopy width across 2 perpendicular axes at the widest point were measured using a Vernier calliper for smaller measurements and measuring tape for larger measurements. At the thirty cm height chosen to measure trunk circumference, there tended to be multiple branches because the first jorquette was low to the ground (10-45 cm). Consequently, multiple diameter measurements were taken and the sum of the cross-sectional area of all wood at that height was derived to be used as an independent variable. This reasoning is based on Leonardo da Vinci's rule which states that "the sum of the cross-sectional area of all tree branches above a branching point at any height is equal to the cross-sectional area of the trunk or branch immediately below the branching point." More recently, this rule was tested against biomechanical models by Minamino and Tateno (2014) who found that the models agreed with da Vinci's rule when the branching angles of daughter branches were small (which was the case in this study as basal growth was orthotropic).

Canopy area is calculated from canopy widths using the formula of a symmetrical closed curve (oval shape) (**equation 1**)

$$Canopy\ Area = \pi \times r1 \times r2 \quad (1)$$

Where *r1* is the canopy radius along the row of trees and *r2* is the canopy radius perpendicular to r1

3.5.2. Model Selection and Application

Various linear regression models were made with the variables above to predict total biomass and the biomass of important tree components. Both transformed and non-transformed natural logarithms were used to find the best fitting model. Models were selected based on (1) highest R^2, (2) Highest adjusted R^2 (R^2 adj), (3) lowest root mean square error and (4) practicality (the model needs to be easily applicable and realistic for future use).

Pearson correlation coefficient was calculated between total and component biomass for each age and for all ages combined and the variables to identify the individual predictive strength of each variable.

The model was then applied to ten twenty-one-year-old trees to predict the total biomass of older trees than what were sampled in this study.

3.6. Statistical Analysis

All statistical analyses were done using the SPSS software programme. Data was analysed using ANOVA with significance expressed using Fishers LSD with a significance level of $p<0.05$. Where necessary, standard deviation or coefficient of variation is displayed alongside the mean of a dataset. Box plots are used for graphical illustrations of the data's distribution with outliers being removed from the analysis but still shown on the plot. Scatter plots are used to show correlations in SPAD data with N and Mg. Pearson's correlation Linear regression models are used with either transformed or untransformed variables to establish allometric equations.

4. Results

4.1. Tree Biomass

After destructive analysis of 15 trees, the results show that the average total above and below-ground biomass per tree increases with age from 0.6 kg per one-year-old tree, to 11.4 kg per 6-year-old tree and 27.5 kg for 11-year-old trees (**Table 7**). The total biomass per ha in **Fig.10** corrects for differences in planting density but offers a very similar pattern; In one ha of intensively grown cocoa, you can expect 900, 17000 and 31000 kg of dry biomass for 1, 6 and 11-year-old trees respectively.

Table 7: Mean total and component dry biomass per age and SD of the mean.

Age	Component Dry Biomass (kg/tree)											
	Lateral Roots		Leaves		Shoots		Tap Root		Base + Branches		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.04	0.02a	0.3	0.1a	0.09	0.05a	0.05	0.02a	0.1	0.03a	0.6	0.2a
6	1.3	0.2b	1.8	0.4b	0.5	0.1b	1.3	0.4b	6.6	1.8b	11.4	2.2b
11	3.3	0.6c	2.0	0.7b	0.6	0.1c	3.7	0.7c	17.8	2.7c	27.5	3.5c

Significant ($p<0.05$) differences after one-way ANOVA for Total and Component Dry Biomass are denoted by different letters according to LSD

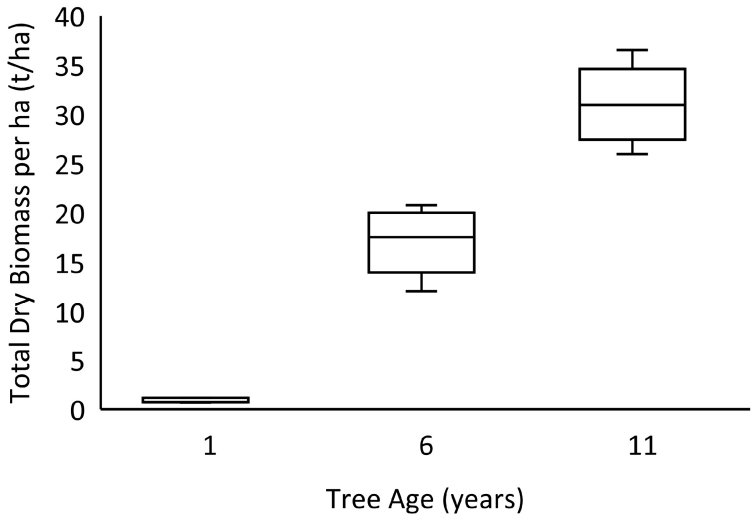

Figure 10: Calculated total dry biomass per hectare of 1, 6 and 11-year-old cocoa trees

4.1.1. Biomass Allocation

The dominant component in juvenile cocoa trees were the leaves (51% of total tree biomass) whereas in mature trees the woody component (base and branches) made up the majority of the tree's biomass (58% for 6-year old trees and 65% for 11-year old trees) (**Fig. 11**). The proportion of biomass allocated to leaves and shoots decreased drastically between 1 and 6-year-old trees but any further alterations to 11-year trees were small in comparison. The opposite is true for branches which were present in increasing amounts in the older trees. The tap root and lateral roots were present in relatively equal proportions regardless of tree age. Mature trees had a slightly greater proportion of roots relative to the 1-year-old trees where roots made up just 15% of the total biomass.

In absolute terms (**Table 7**), leaf biomass increased between 1 and 6-year trees but no further significant differences were identified between 6 and 11-year trees. For the biomass of the other components and for the total biomass, significant increases with tree age were observed.

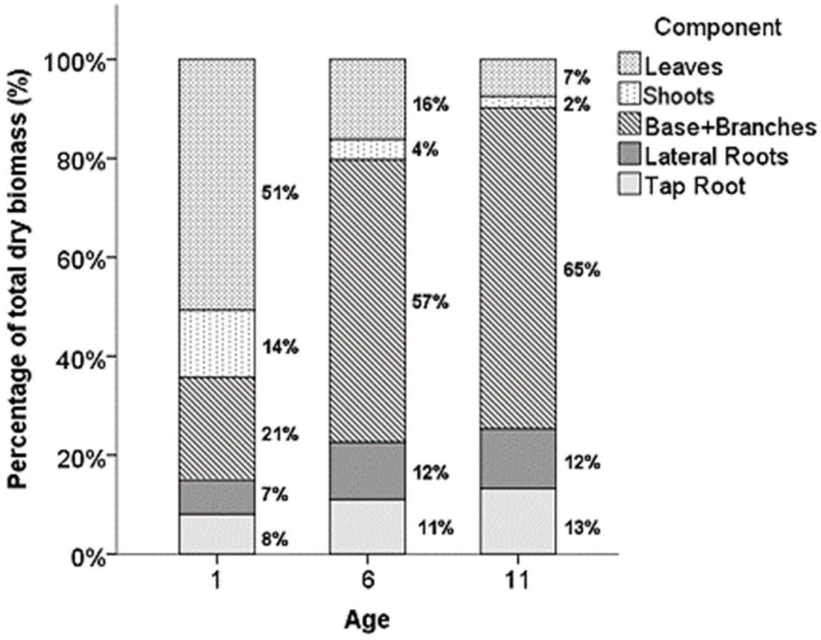

Figure 11: Dry biomass allocation to the various tree components expressed as a percentage of the total biomass for each age

4.1. Canopy Position Analysis

For this section the 1-year-old cocoa trees were not considered because their leaves were generally smaller than the other ages and the middle canopy section was not measured for these leaves. For the remainder of the leaves, regardless of canopy position, there was great variability in leaf area (**Fig. 12**). The mean leaf areas for the three canopy positions were 244cm², 218cm² and 214cm² for bottom, middle and top respectively but no significant ($p<0.05$) differences were found using LSD.

However, **Fig. 13** shows that the trees favour allocation of new leaf production to the top of the tree which receives the greatest amount of incident light. According to LSD, no significant ($p<0.05$) difference was identified between the percentages of current flush leaves on the bottom and middle canopy position, but the top position was significantly different from both.

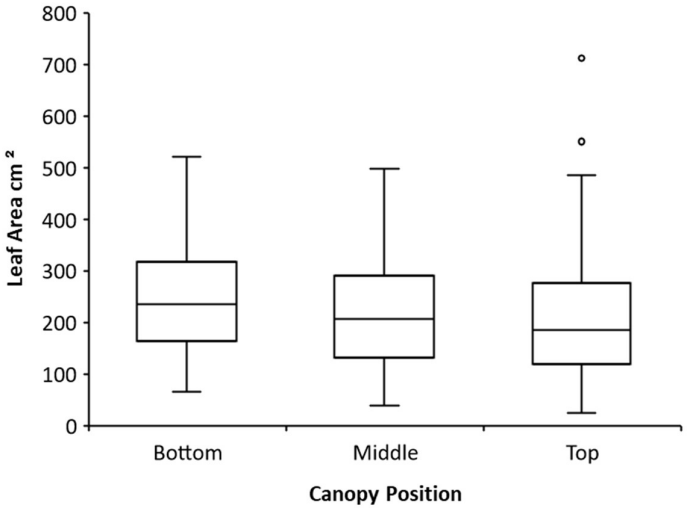

Figure 12: Average leaf area for leaves of the previous flush at various canopy positions

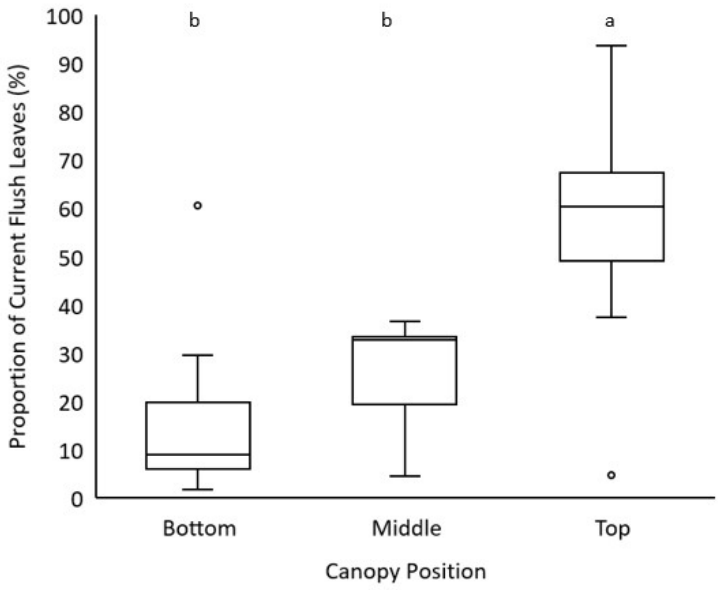

Figure 13: Distribution of current flush leaves to bottom, middle and top canopy positions, as a percentage of total dry biomass of current flush leaves for each tree

4.2. Allometric Relations

4.2.1. Variables

The total biomass and component biomass used in the model as independent variables are listed in **Table 7** and have already been discussed. The values of variables used to predict biomass (**Table 8**) increased proportionately with age, much like tree biomass did. Tree height offered less variation than

the other two variables because within each block, trees were trained to a uniform height. Six-year-old trees had a particularly low coefficient of variation (CV) for tree height and canopy area compared to the other ages because these trees had a hedge-row system and were mechanically pruned with a lot more consistency than the manually pruned fields.

Table 8: Means and coefficient of variation (CV) for each variable.

Variable	Tree Age					
	1		6		11	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Basal Area (cm^2)	13.2	13.8	116.4	33.6	240.6	15.6
Tree Height (cm)	88	10.2	278	6.5	321	8.7
Canopy Area (cm^2)	1.0	22.6	4.1	12.4	9.2	27.4

4.2.2. Allometric Models of Total and Component Biomass

The simplest models to predict total above and below-ground tree biomass of *T. cacao* trees are shown in **Fig. 14**. The best fitting modelled independent variable with total biomass was the sum of basal trunk or branch area which had an R^2 or 0.899 (R^2adj of 0.891 (**Table 9**)). Based on this model, for every 1 cm^2 increase in basal area at 30 cm, there is a 0.11 kg increase in total biomass per plant. The logarithm to base 10 (Log10) of total biomass follows an almost perfect linear relationship with tree height (**Fig. 14D**) with an R^2 of 0.973.

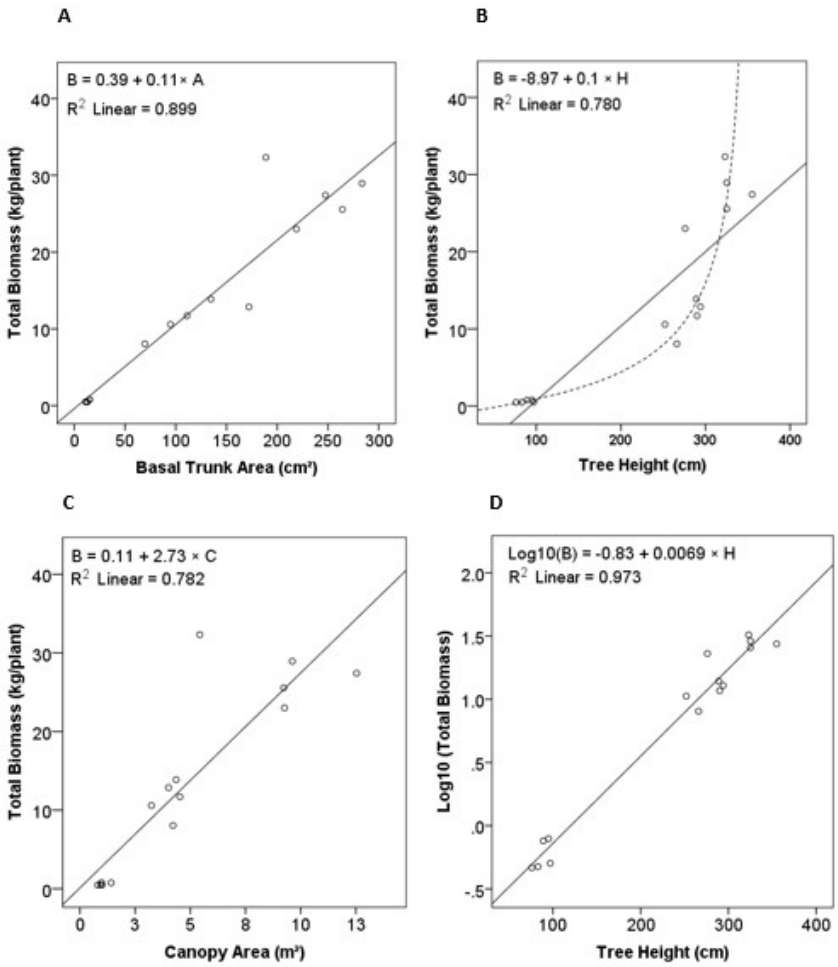

Figure 14: Best linear regression lines (including formula and R^2 value) to predict total tree biomass using Basal trunk area (**A**), tree height (**B**) and canopy area (**C**). Figure **D** shows the linear relationship between Log10 (Total Biomass) and tree height. The dotted line (**B**) is a manually fitted line to indicate the non-linear relationship between tree height and total biomass.

4.2.3. Model selection and Justification

Several allometric equations were made to cater for a variety of applications. **Table 9** contains the best models to predict component biomass. R^2adj values are high ($0.76<R^2adj<0.98$) for all the models and were all significant at greater levels than $p<0.0001$.

Within each age, correlations between component or total biomass and predicting variables were mostly either not related, weakly, or moderately correlated with no significance shown at $P<0.05$ (**Table 10**). When the three age brackets are combined, the predicting variables showed strong positive correlation ($0.75<r<0.9$) with all the biomass components as well as total above and below-ground biomass. All correlations for predicting general tree biomass were significant at $P<0.01$. Because each of the parameters are sufficiently strong predictors for biomass, it is possible to use one independent variable to avoid collinearity. Furthermore, it allowed the freedom to select the

independent variables which seemed more feasible to use in the field and ones which seemed more logical to use based on scientific reasoning behind the intensive farming system. With that said, measuring canopy area in the field is less easy to do than measuring height and average circumference and offers substantial room for error in measurements. Moreover, height and canopy area would be less useful as independent variables because the trees are topped and pruned on a regular basis. This meant that these parameters are relatively constant in older trees regardless of biomass (**Fig. 14B**), and they may vary according to the amount of time since the last pruning. Seeing as most of the pruned material are leaves, using height as a predictor of leaf biomass still makes sense.

Table 9: Best fit models to predict leaf, wood, root and total biomass

Model	R^2	R^2adj	Std Error	p value
Total Biomass				
B = -0.392 + 0.11 × A	0.899	0.891	3.8	<0.0001
log10(B) = -0.768 + 0.01 × A + 0.006 × H	0.976	0.972	0.13	<0.0001
Leaves				
B = -0.37 + 0.008 × H	0.802	0.786	0.42	<0.0001
Log10(B) = -0.836 + 0.004 × H	0.894	0.886	0.14	<0.0001
Base Branches & Shoots				
B = -0.758 + 0.076 × A	0.906	0.898	2.5	<0.0001
Log10(B) = -1.33 + 0.001 × A + 0.007 × H	0.975	0.97	0.15	<0.0001
Roots				
B = -0.165 + 0027 × A	0.783	0.766	1.5	<0.0001
Log10(B) = -1.702 + 0.002 × A + 0.007 × H	0.968	0.963	1.6	<0.0001

B: Biomass, A: cumulative trunk/branch are at 30 cm, H: height, R^2: coefficient of determination, R^2adj: adjusted coefficient of determination, SE: standard error of the regression line,

This is confirmed as leaf biomass is most strongly correlated with height (**Table 8**). As previously mentioned, the logarithm to base 10 (Log10) of total biomass follows an almost perfect linear relationship with tree height (**Fig. 14D**)**.** As a result, using this transformation in any model improves its fit. However, using height as a predictor becomes unviable in older trees where trees are trained to a constant height.

Most tree biomass is found in the woody component so sum of base area at 30cm is prioritised as being the most useful predictor of tree biomass in the allometric equations. But, because of their accuracy, tree height models are included but should be used with caution.

Table 10: Pearson Correlation Coefficient (r) between roots (tap and laterals), wood component (base, branches and shoots), leaves and total above and below-ground biomass with tree Height, sum of basal trunk or branch area at 30cm above ground, and canopy area for each sampled age and in general for all sampled trees combined

	Age											
Component	**1**			**6**			**11**			**General (all ages)**		
	Height	**Basal Area**	**Canopy**	**Height**	**Basal Area**	**Canopy**	**Height**	**Basal Area**	**Canopy**	**Height**	**Basal Area**	**Canopy**
Roots	0.559	0.778	0.415	0.198	0.698	-0.41	0.372	-0.530	-0.687	0.833**	0.885**	0.809**
Base, branches & stems	0.326	0.990**	0.665	0.696	0.716	0.337	0.323	0.081	-0.342	0.862**	0.952**	0.889**
Leaves	0.52	0.807	0.591	0.667	0.857	0.151	0.408	-0.299	0.122	0.895**	0.760**	0.754**
Total above and below ground	0.512	0.929*	0.635	0.702	0.851	0.21	0.52	-0.262	-0.514	0.883**	0.948**	0.884**

*: significance at $p < 0.05$, **: significance at $p < 0.01$

4.2.4. Model application

Sum of base area at 30 cm, height and canopy area were also measured for ten twenty-one-year-old trees to apply the model and make an estimation of tree biomass in older trees. Based on the reasoning above, the simplest linear model using only sum of basal area was used (**equation 2**).

$$B = -0.392 + 0.11 \times A \quad (2)$$

Where B is total biomass and A is the sum of basal area of the trunk or branches at 30cm above ground.

Based on this model, the data, and the tree dimensions recorded in the field, the twenty-one-year trees average 20.9kg per plant (**Fig. 15 A**) with a standard deviation of 5.3. This is slightly lower than the measured total biomass of eleven-year-trees. However, once multiplied by the plant density per block (**Fig. 15 B**) the calculated total biomass per ha is visually only slightly higher than that of eleven-year trees, indicating a plateau in total biomass at this point.

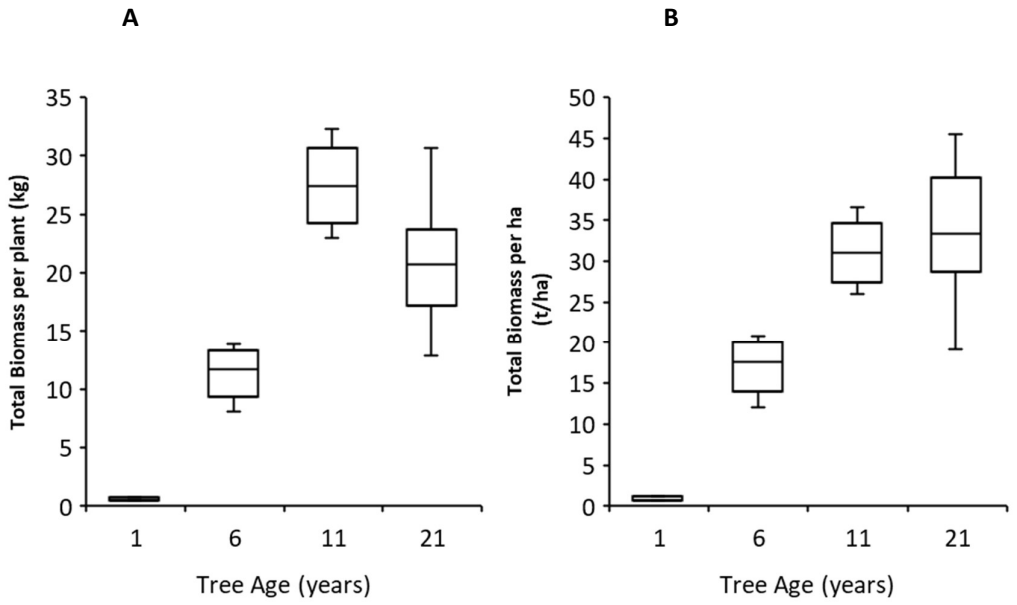

Figure 15: Total biomass per plant including modelled biomass of 21-year-old trees from two separate blocks (A) and calculated total biomass per ha based on plant density for each block (B)

4.3. Nutrient Analysis

4.3.1. Total Nutrient Uptake

The total nutrient allotment to roots, wood and leaves per ha is shown in **Table 11**. For all ages, the macronutrients present in highest quantities are N, K and Ca. At maturity (eleven-year trees), a ha of the intensely farmed cocoa locks away 512, 486 and 392 kg respectively for N, K and Ca. The remaining macronutrients, P, Mg and S were accumulated amounted to 73, 86 and 46 kg/ha respectively. As expected, micronutrients were taken up in substantially smaller quantities with B and Mn being accumulated in highest quantities at all ages. The oldest cocoa trees in this study immobilised 0.84 kg of B and 4.7 kg of Mn per ha.

Table 11: Total macro and micronutrient accumulation (excluding fruit) per ha by 1, 6 and 11-year cocoa trees

Age	Macronutrients (kg/ha)					
	N	P	K	Ca	Mg	S
1	15	1.8	15	11	2.5	1.2
6	269	32	270	217	47	26
11	512	73	486	392	86	46
Age	Micronutrients (kg/ha)					
	B	Cu	Fe	Mn	Zn	
1	0.02	0.0003	0.002	0.1	7.5×10^{-9}	
6	0.40	0.0004	0.003	2.4	1.1×10^{-8}	
11	0.84	0.0003	0.003	4.7	8.8×10^{-8}	

Macro and micronutrient uptake rate in this study were found to be:

N > K > Ca > Mg > P > S > Mn > B > Fe > Cu > Zn

Because the nutrient concentrations do not differ substantially from each other for each component, nutrient allocation is much the same as biomass allocation.

4.3.1. Component Nutrient Concentrations

The macro and micronutrient concentrations of the tree components are illustrated in **Fig. 16** and **Fig. 17**. Seeing as the leaf flush analysis showed no difference in nutrient concentrations, the leaf concentrations below are a combination of all leaf nutrient concentrations. Also, no fruit or husk samples were taken from the 1-year lots as they were not bearing fruit. As a result, the sample sizes are different for these components. The data shows all tree ages combined because there were no differences between ages.

As with the leaf flush analysis, there is a wide spread of data points with many outliers in the box-plots. It is difficult to say with confidence that there are any obvious differences in nutrient concentration between the components.

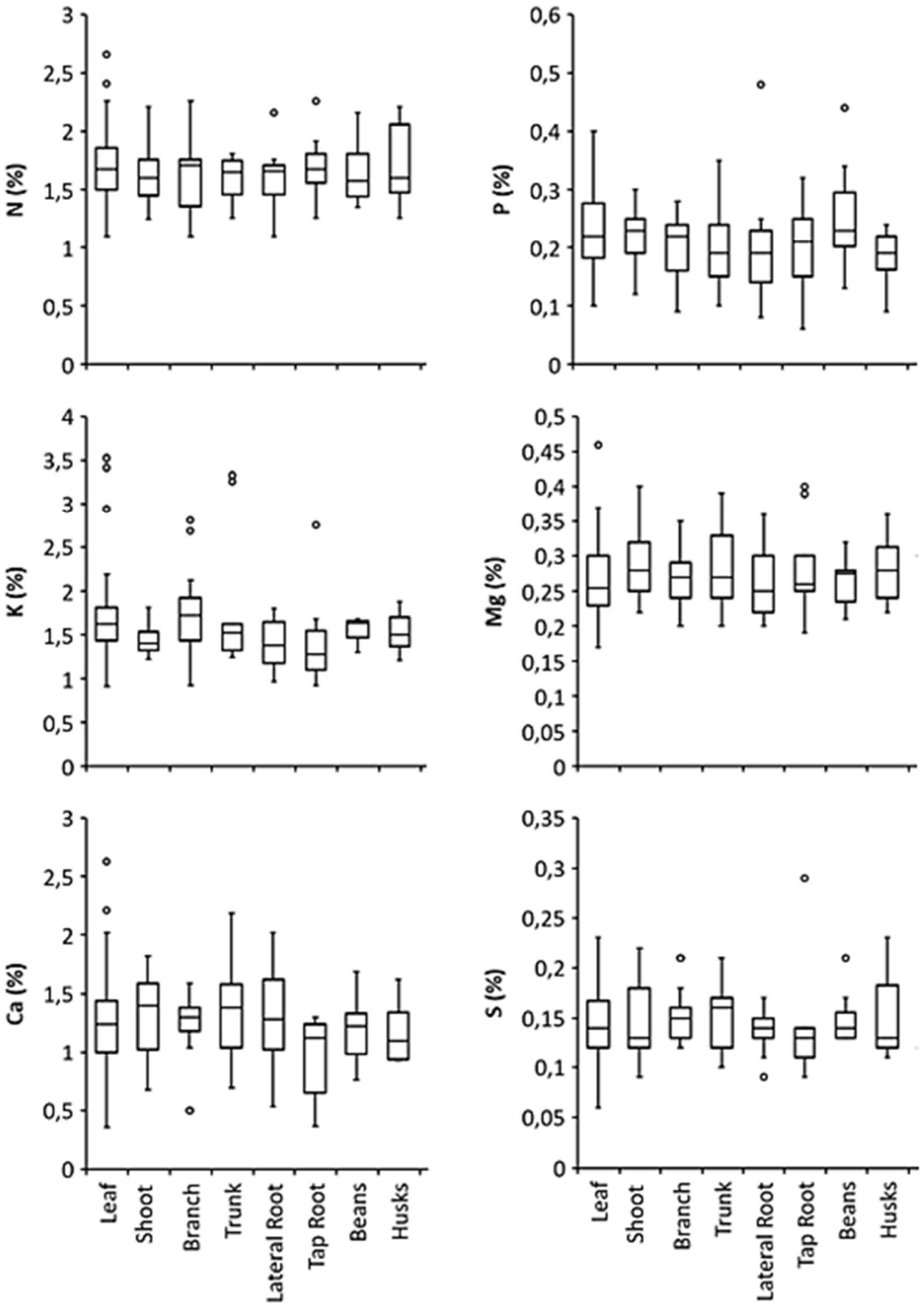

Figure 16: Macronutrient concentrations of various tree components

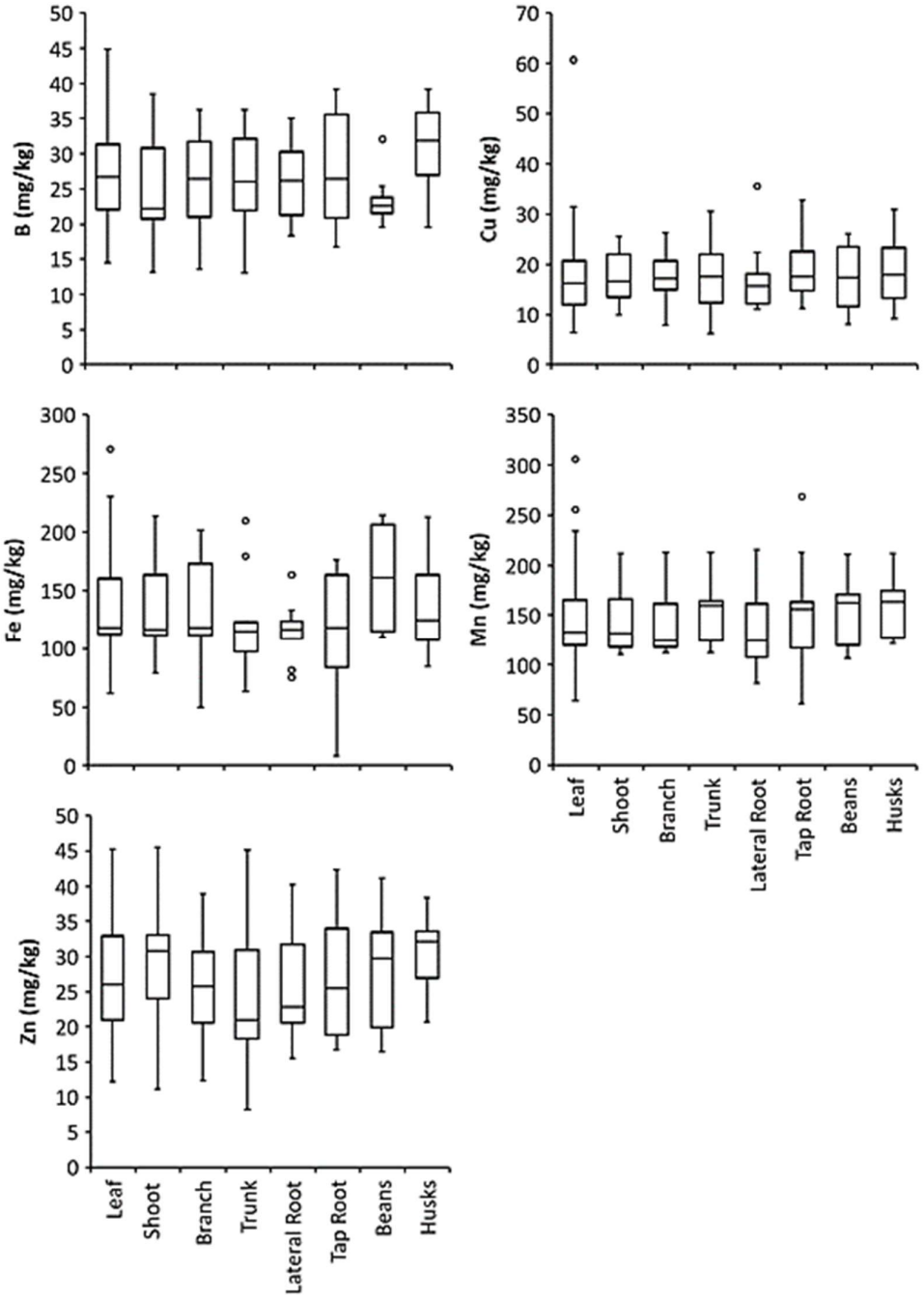

Figure 1712: Micronutrient concentrations of various tree components

4.3.2. Foliar Nutrient Analysis of Leaves in Senescence, Leaves of the Current Flush and Leaves of the Previous Flush

A leaf flush analysis was done to observe nutrient mobilisation to young leaves and some field observations were made that are useful to know when approaching this section. Firstly, the amount

of leaves in senescence in all the sampled trees was very small (usually 5 to 10 leaves) with an average dry weight per mature tree of 118 g. Secondly, a pruning or topping event was shortly followed by a breakout of a new flush. In **Appendix A** the tree that was pruned a week prior to the photo has substantially more new-flush leaves than both the tree that had not been pruned recently and the tree that had been pruned a few hours before the photo was taken. Unfortunately, the time between pruning and/or the onset of a new flush of leaves and the sampling for trees was not always uniform. As a result, within trees, the flush was uniform, but between trees it was not. **Appendix B** shows the different maturity levels of new flush leaves which were sampled in the experiment.

Samples were taken for analysis of Leaf macro (N, P, K, Mg, Ca and S) and micro-nutrient (B, Cu, Fe, Mn and Zn) contents of three flush groups, namely; leaves of the current flush (LCF), leaves of the previous flush (LPF) and leaves in senescence (LS). The results are summarised in **Fig. 18** (macronutrient analysis) and **Fig. 19**(micronutrient analysis). The results indicate little or no variation in nutrient concentration between the flush groups. The macronutrients present in highest concentrations are N, K and Ca in that order. Fe and Mn occur in the highest concentrations of micronutrients in the leaves. In all the analyses, the box-plots show a large spread of data (high variation) with many outliers.

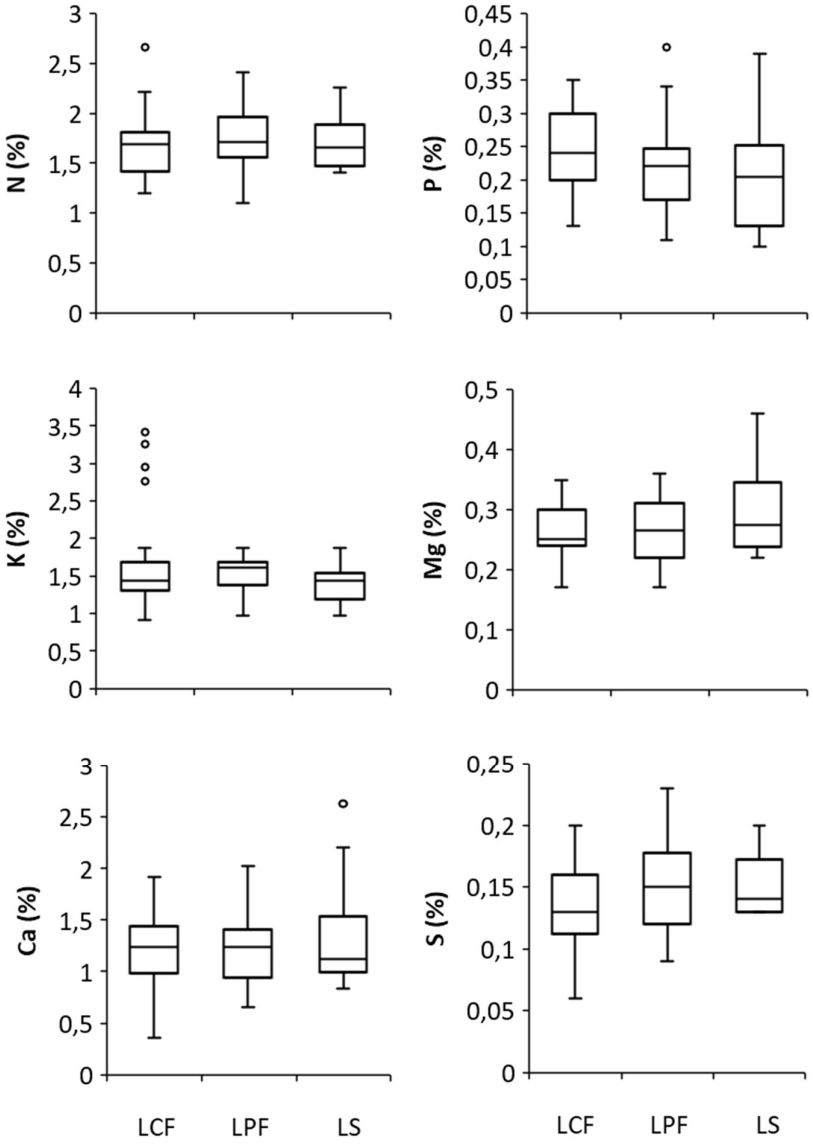

Figure 18: Macronutrient concentrations of leaves of the current flush (LCF), leaves of the previous flush (LPF) and leaves in senescence (LS)

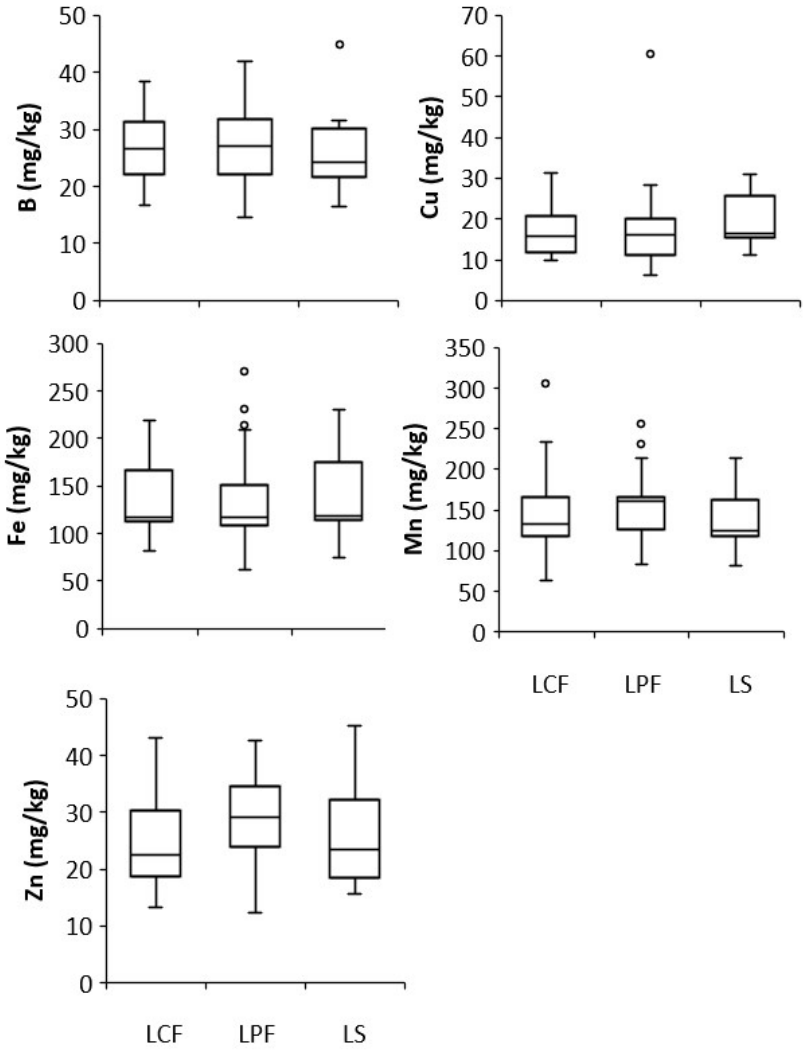

Figure 19: Micronutrient concentrations of leaves of the current flush (LCF), leaves of the previous flush (LPF) and leaves in senescence (LS)

4.3.4. SPAD Calibration Curves

For a useful calibration curve to be established which can predict leaf N or Mg, strong correlations are needed between individual SPAD and N or Mg measurements. However, SPAD units do not appear to have any sort of correlation with either N or Mg in **Fig. 20.**

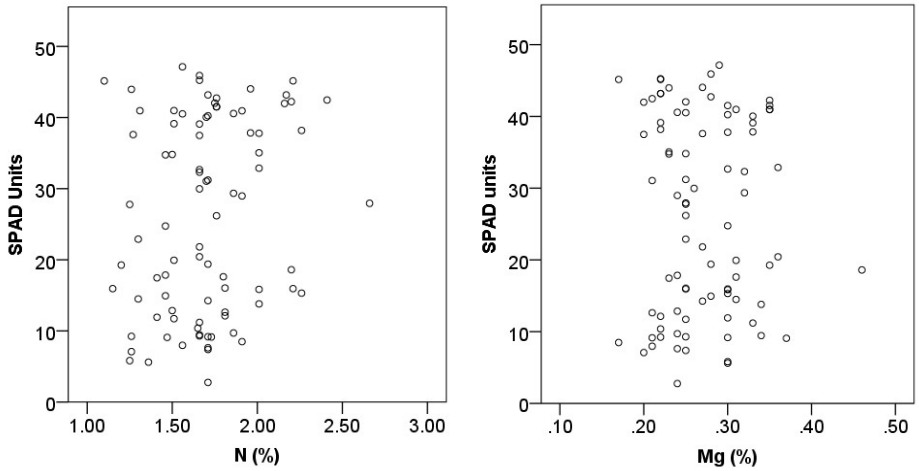

Figure 20: Scatter plot showing the relationship with SPAD units against N (left) and Mg (right) (n = 86)

5. Discussion

5.1. Biomass

5.1.1. Total Biomass

T. cacao trees are known to reach a plateau in total biomass at a certain point. No plateau had been identified from the trees sampled in this study. Even up till 11-year trees, growth in biomass still seemed linear. By applying the allometric models created in section 4.2. to some 21-year trees, the estimations show that 21-year trees do not have a greater biomass than 11-year trees do. Thus, revealing the stability of total biomass in mature cocoa trees. In the studies by Thong and Ng (1980) and Calvo Romero (2018), the total biomass had already slowed down before they had reached 5 years. In this study on an intensive plantation however, the plateau was reached at or near the ages of 11-years. Furthermore the maximum weight of mature (11-year) trees in this study was substantially lower than the weight of trees in the studies by Calvo Romero (2018) and Thong and Ng (1980).

It is common practice to intercrop young cocoa trees (particularly with banana or plantain) to provide them with sufficient shade to ameliorate photoinhibition (Acheampong et al., 2009). A possible reason for delay in reaching a so called “plateau” in growth is that the trees in this study were established as a monocrop, meaning that despite the higher density, young plants were exposed to high levels of

incident light thereby resulting in photoinhibition of the light harvesting complex, hindering the trees photo-assimilation for growth (Almeida and Valle, 2007). Whether the prolonged allocation of assimilates to biomass growth affects fruit production in the early production years (tree ages 5 till 7) is unclear but intensive farms could benefit from using a temporary intercrop such as banana or plantain if that is the case. Another possible reason could be that the 6-year block had a hedgerow system with tractor lanes after every second row which the 11-year block did not have. Other noteworthy differences between the two blocks which should not be ignored are some minor variances in soil types (**Fig. 7**), different irrigation plans (drip system in 6-year trees vs microjet system in 11-year trees) and mechanical pruning for 6-year trees vs manual pruning for 11-year trees.

Isaac et al. (2007) showed that 8-year *T. cacao* trees grown in the shade accumulate twice as much biomass as trees grown in full sun when the density of trees is uniform. Boyer (1973) had contrasting data, with 30-year trees grown in full sun accumulating 1.5 times as much biomass in the wood component than moderate shade trees at the same density (**Table 1)**. This however is not the generally described trend (Somarriba and Beer, 2011). In a hectare of intensely grown mature (11-year) cocoa in this study, approximately 30 to 35 tons of dry biomass were accumulated (excluding shade trees) with each tree averaging 27.5kg. This is about half of what was shown by trees of a similar age in the study by Calvo Romero (2018). Thong and Ng (1980) showed that a mature cocoa tree weighed roughly 40 – 50 kg which is also substantially higher than the trees in this study. In another study, Aranguren et al. (1982) found that 30-year trees in an agroforestry system accumulated just 17.2kg per tree (17.1 tons per ha), far less than any of the abovementioned studies.

It is difficult to justify comparisons between the studies in **Table 1** and my results because it is difficult to pinpoint the defining factors in most of their results. Variations in site conditions, management practises, varieties etc are all probable. No studies exist such as this one, where biomass was measured for intensively grown cocoa, so these results serve as a benchmark for further studies of this kind. In theory, monoculture crops should be grown at a higher density than agroforestry crops to provide self-shading for the trees. If the limiting factors such as the nutritional needs are accounted for, could the higher density counteract for the positive effect on growth by shade trees? By these results it seems unlikely because the biomass is low, but this could be because of more thorough pruning practises along with a variety of other explanations mentioned above.

There is a serious mismatch in the classification of cocoa systems. For example, what one author may describe as an agroforestry system, another would define as a monoculture making it very difficult to compare results from previous studies. Moving forward, better categorisation of farming systems need to be made.

5.1.2. Biomass Allocation

As expected, the proportion of biomass in the leaves was substantially higher in young trees as compared to mature trees, in accordance with research done by Thong and Ng (1980). This shows the importance of maximising the photosynthetic potential of young trees to acquire C for tree establishment before reproductive maturity of the trees is reached. The decreasing proportion of leaves in cocoa trees goes hand in hand with an increase in biomass partitioned to the wood component as cocoa trees progress from juvenility to maturity.

Distribution of biomass between the above and below-ground component did not change much between any of the tree ages. Much like the study by Thong and Ng (1980) and Bastide et al. (2009), the proportion of biomass in the roots remained within 15% and 25%, with an equal distribution between tap and lateral roots, showing the importance of both anchoring the tree by the taproot and accumulating water and nutrients by the lateral roots.

The woody component (base and branches) is an important storage organ and flowering site for fruit production. In this study, the base and branches accounted for most of the biomass in mature cocoa trees, presenting similar proportions to Thong and Ng (1980) and Bastide et al. (2009) despite heavy pruning.

5.1.3. Allocation According to Canopy Position

Average leaf area per showed a tremendous amount of variability in each canopy position making any differences difficult to prove statistically. This could possibly be explained by carbohydrate limitations to a certain proportion of the leaves within a flush, resulting in growth limitation (Machado and Hardwick, 1987). However, this cannot be proven. Using SLA instead of leaf area is more common in literature and would have been more useful but unfortunately, due to the complexity of the experimental design (leaf samples were used for nutrient analysis, SPAD analysis, and biomass analysis) and the inability to dry samples on location, it was not possible to obtain this data. In a brief analysis of the local pruning procedure, an average of 1.93kg of fresh leaf tissue was removed per tree at every pruning event. Pruning allows a more even light distribution throughout the canopy, promoting light penetration to flowering positions on the lower branches and encouraging leaf growth for CO_2 consumption. The rigorous pruning schedule would mean that there was greater amount of "sun flecks" reaching middle and lower canopy leaves.

Despite this, a substantially greater proportion of new leaf production was allocated to the top section of the canopy, so by means of volume, more nitrogen and other resources would be allocated to the upper canopy where more incident light would be expected.

5.2. Allometric Relations

Various allometric models were generated to predict component and total tree biomass with a great deal of accuracy. Strong correlations existed between each predicting variable and biomass allowing simple and effective measurements with only one independent variable to be made. That said, the most useful variable in the models was the sum of basal trunk/branch area at 30 cm because it is the easiest to measure, and it offers a greater accuracy than either of the other untransformed variables. The studies in **Table 3** agree with this study in that the trunk or branches act as the most suitable estimators of biomass. This makes sense as most of the biomass is found in these components. Models which incorporated height as a transformed independent variable further improved the fit but should be used with care as trees at maturity were trained at a certain height meaning that biomass would be overestimated by small increases in tree height. Using more independent variable also increases the chance of collinearity. Unlike any previous study, this study stands out in that the prediction of component biomass is possible making non-destructive estimations of component biomass possible.

One limitation for the models created in this study is the relatively small number of trees harvested (5 for each of the 3 tree ages sampled). For example, Toknok (2011) sampled 35 cocoa trees (presumably all mature) and Smiley and Kroschel (2008) sampled 45 cocoa trees whilst establishing their allometric equations. This shows in the results as the Pearson's correlations within each age group in this study are low and don't have significance compared with a combination of all the sampled trees (**Table 10**). This means that the model may not be applicable for comparing trees within the same age or with very similar independent parameters. Furthermore, these models are specific to intensive cocoa systems and their application to varieties other than CCN-51 may be limited but this needs to be tested.

5.3. Nutrient Dynamics

To see how nutrient allocation and immobilisation compares to existing data, macro and micro-nutrient concentrations were measured for each tree component. The results were somewhat contrary to expectation given that the trees are grown in an intensive system. The main discrepancies in the data are: firstly, the nutrient status of the tree appears to be low regardless of the tree component. Considering the bi-monthly fertilisations, one would expect nutrient concentrations to be high. Secondly, there appears to be somewhat equal concentrations of nutrients across all components. This is contrary to the original hypothesis that leaf and fruit tissue would be nutrient dense than woody tissue as described by Dossa et al. (2008). Thirdly, there was no nutrient remobilisation from old leaves to leaves in the current flush. Finally, the hypothesis that K would be

higher in the husks was also not found to be true. All these inconsistencies exist even though the yields on the farm are still high (up to 3.8 t/ha in the highest yielding lots).

Many steps were taken to verify the results. Some N and K samples were redone with a WEPAL reference sample included. Both repetitions returned the same results. To ensure labels weren't mixed up, 105 random samples were observed in the lab to ensure that samples that were hypothetically woody tissue in the data, were. In every case, they did return the correct tissue type. The lab also reported some unpublished data for leaf tissue analyses from the same farm and some surrounding farms that also verify the results found in this study.

This leaves the question as to why the results are different to what is expected and is the "text-book" knowledge of cocoa nutrition in question? Perhaps these unexpected results are because of the intensive farming system used or the climate, cultivars and soils found in Ecuador. *T. cacao*.

Unfortunately, performing soil analyses was not possible. Many factors within the soil can influence nutrient uptake. These include soil depth, CEC, fertility, moisture status, structure, pH, antagonisms, etc. The criteria for tree selection are that they should be free from pests and diseases but besides visual analysis, no tests for nutrient uptake limiting soil diseases and nematodes were done. The nutrient availability is assumed to be high because fertilisers are applied frequently. However, without any soil analyses, this assumption cannot be proven and nutrient availability to the plant is unknown.

In terms of total nutrient uptake which is dependent on component biomass and corresponding nutrient concentrations, the order of immobilisation of macronutrients was N > K > Ca > Mg > P. This is contrary to the results by Ling (1984) and Thong and Ng (1980) who both showed that uptake was in the order of K > N > Ca > Mg > P. Alpizar et al. (1986) on the other hand presented an order of N > Ca > K > Mg > P. At maturity (eleven-year trees), a ha of the intensely farmed cocoa in this case locks away in the trees biomass 512, 486 and 38 kg respectively for N, K and Ca. Compared to the studies by Ling (1984) and Thong and Ng (1980) there is more N in the system, less K and comparable amounts of Ca.

A possible explanation for uncertainty in the order of importance between this study and the ones mentioned above is that K and Na can be used interchangeably by the cocoa plant. Gattward et al. (2012) showed that in the soils where K was partially replaced by Na, that plant performance (photosynthesis and WUE) improved and concluded that K nutrition of *T. cacao* can be partially replaced with Na. Na is often overlooked and is very seldom included in plant nutrient analyses.

The same theory could explain the lower than expected K levels in the husks of the fruit. Perhaps, some of the important roles of K in the husks such as enzyme activation, carbon assimilation,

translocation of sugars and proteins, etc could have been replaced by Na. With that reasoning it could be worth repeating some of the analyses for Na concentration to see whether Na + K is higher in the husks than in other components.

Like many plants, *T. cacao* is known to remobilize nutrients from old to young leaves(Aerts, 1996; Almeida and Valle, 2007). However, contrary to expectation, no significant differences were identified between nutrient concentrations in senescing leaves, leaves of the previous flush or leaves in the currents flush. Flushing of the sampled trees was clearly not uniform. This is likely because pruning was observed to induce a new flush and the times of pruning for each block were different, allowing varied levels of new flush development. **Appendix A** illustrates the effect of pruning on the trees and **Appendix B** shows the range of development of leaves of the current flush that were measured. From the latter, it is evident that more recently flushed leaves appear red and with time they develop chlorophyll pigments. Unlike most plants found in temperate climates where chlorophyll concentrations reach a peak before the leaf is fully expanded, cocoa leaves synthesise chlorophyll slowly during expansion, reaching a peak well after expansion is completed (Abo-Hamed et al., 1984). These lacks in consistency in flush development stage do not explain the absence of remobilisation from leaves in senescence. It could however explain some of the variation in nutrient concentrations (particularly in leaves) as various trees were in different stages of flushing which could affect the nutrient dynamics in the tree and the source-sink relationships of the leaves.

Finally, this study does not account for temporal changes in nutrient flows in the system. The *T. cacao* trees in the sampled farm experience seasonal variation and at the time the fieldwork was done, the trees were in an unproductive phase. In perennial systems, it would be more interesting to look at the long-term trends in nutrient dynamics. As the study farm changed ownership and therefore management practises two years prior to this study taking place. The ability of fertilisation and management practices to take effect on nutrient dynamics could take 4-5 years.

The results are unexpected which brings forth many interesting anomalies regarding cocoa nutrient dynamics. This study should encourage similar research, possibly in a more controlled way so that uncertainties such as mentioned in the paragraphs above can be accounted for.

5.3.1. SPAD Calibration

An attempt was made to establish a SPAD calibration curve which would make foliar N and Mg sampling in the future much simpler. However, leaf chlorophyll measurements (SPAD units) failed to show any correlations with either N or Mg. As a result, a SPAD-nutrient calibration curve is not feasible with this data. The reasoning behind a calibration curve is that in theory, much of the N and Mg in leaves is locked away in the chlorophyll molecules. However, it is not always the case that leaf

chlorophyll is related to leaf Nitrogen. For example, Yadava (1986) found that for many horticultural crops, SPAD units showed strong correlations with leaf chlorophyll but not with leaf N. Although the reasons for the variable performance of SPAD-meters in predicting leaf nutrient status are unclear, there are speculations that there is greater complexity in the nutrient dynamics of perennials (Loh et al., 2002). In their early stage, leaves have low SPAD measurements because of an evident lack of chlorophyll pigment and the masking of chlorophyll by higher levels of other pigments such as anthocyanins (leaves appear red or light green)(Lee et al., 1987). Moreover, Lawlor et al. (2001) write that in some cases as little as 2% of the total leaf N can be contained in chlorophyll molecules so in these cases, SPAD would be a poor indicator of the leaf nutrient status as we see in these results. Therefore in agreement with Calvo Romero (2018) and as per the hypothesis, establishing a SPAD calibration curve is not possible for *T. cacao.*

6. Conclusion

This is the first known study of its kind to be carried out on an intensive *T. cacao* farm where biomass and nutrient dynamics of cocoa trees were measured to explore the relations and physiological trends which developed. 1, 6 and 11-year-trees were sampled and allometric equations were made to predict tree biomass using transformed and untransformed parameters such as the cumulative basal area at 30cm above ground level. These equations were applied to 21-year-old trees to estimate the trends in biomass at older ages and the sampled trees showed that there was no further increase in biomass after age 11. However, total biomass of the trees did continue to increase after age 6 which is contrary to the general trends discovered by previous authors. This study showed that leaf nutrient mobilisation was minimal. Component nutrient concentrations did not differ which is an anomaly when compared to existing studies. An attempt was made to construct SPAD calibration curves with Mg and N based on the theory that leaf chlorophyll is made up of large amounts of these nutrients, but no correlations were found between SPAD units and either N or Mg.

This study took place during a slow period in fruit production, so it would be interesting to see if the nutrient dynamics and other findings in this study would be the same during a heavy fruiting period. Similarly, tracking nutrient and biomass development of cocoa trees with time has not been done in depth by any researchers and would add valuable insights to cocoa nutrition studies.

Some unexpected results were reported for both nutrient and biomass allocation and immobilisation. Some possibilities were proposed which could possibly explain these irregularities but clearly, we are very far from fully understanding *T. cacao* in scientific and agricultural contexts. This study paves the way for more research to be done in intensive cocoa farms to verify these results and to delve deeper into the reasons for these results. This study and any further studies of its kind will be beneficial to

small-scale and commercial farmers alike. They will act as a major stepping stone to achieving sustainable intensification which the industry needs to combat forest degradation and to boost social welfare of cocoa farmers.

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8. Appendix

8.1. Appendix A

Figure 21: Cocoa trees immediately after pruning

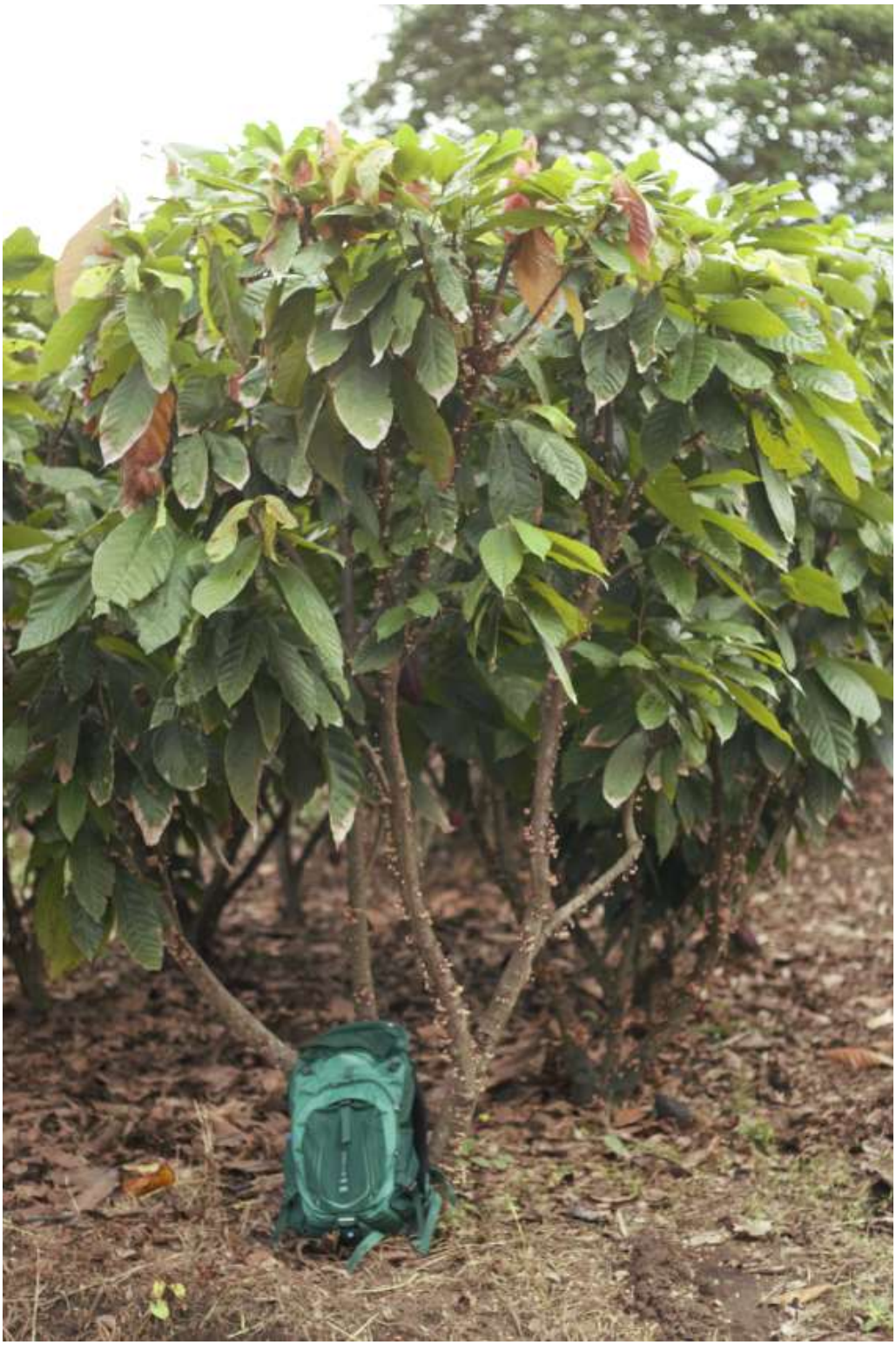

Figure 22: Cocoa trees before pruning

Figure 23: Cocoa trees approximately 1 week after pruning

8.2. Appendix B

Figure 24: Current flush leaves shortly after bud break

Figure 25: Current Flush leaves after bud break but before full expansion of the leaves and production of chlorophyll molecules

Figure 26: Current flush leaves approaching full expansion and having developed green chlorophyll molecules