Impact of agro-forestry systems on coffee yield, coffee plant morphology, physical and chemical attributes of green coffee beans and aroma generation of roasted coffee beans

by

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ABSTRACT

A long experiment has been established since 2000 at CATIE (Tropical Agricultural Research and Higher Education Center), Turrialba, Costa Rica. Twenty agro-forestry systems with different shade types and managements (organic and non-organic) consisting of an incomplete randomized block-design with shade tree as main effect and subplots represented by management were set up. The mean 13-year yield, bienniality index (BI) of coffee yield, pruning, shade cover and morphology (height, diameter, total branches and productive branches) of coffee plants in these agro-forestry systems were measured. The effects of different managements and shade types on the physical and chemical attributes of green coffee beans and aroma and colour generation of roasted coffee beans were investigated. The organic management always showed the more stable production, while the coffee yield and morphology were always worse under the intensive organic (IO) management than under the intensive conventional (IC) management. Comparing with the moderate conventional (MC) management, the intensive organic (IO) management did not only have a similar or even higher productivity, but it also had higher total lipid and protein in the green beans and a stronger ability to generate more flavour and colour. The full sun system had a higher total coffee yield and bienniality index (BI) of coffee yield, green bean density and total protein content and greater flavour generation and colour after roasting. Comparing with the timber system, the service system did not only have the higher coffee yield and better coffee plant morphology, but it also produced green beans with higher total protein and roasted beans with the more flavour and colour. Comparing with the non-legume shade tree, the legume shade tree

only influenced the total protein content of green coffee beans and further improved the performance of flavour and colour in the roasted coffee beans.

Keywords: Agro-forestry systems; Shade trees; Organic management; Coffee yield; Coffee morphology; Coffee bean density; Bulk density; Moisture content; Roasting loss; Minerals; Lipids; Fatty acids; Carbohydrates; Protein; Caffeine; Aroma generation

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LIST OF ABBREVIATIONS

ANOVA Analysis of Variance

ATP Adenosine Triphosphate

BCA Bicinchoninic Acid

BI Bienniality Index of Coffee Yield

BSA Bovin Serum Albumin

C *Chloroleucon eurycyclum*

CATIE Centro Agronómico Tropical de Investigación y Enseñanza, Tropical Agricultural Research and Higher Education Center

CE *Chloroleucon eurycyclum* and *Erythrina poepiggiana*

CoA Acetyl-coenzyme A

CT *Chloroleucon eurycyclum* and *Terminalia Amazonia*

D Coffee Root Diameter

E *Erythrina poepiggiana*

ED Electrochemical Detection

ET *Erythrina poepiggiana* and *Terminalia Amazonia*

FAMEs Fatty Acid Methyl Esters

FS Full Sun

GC-MS Gas Chromatograph Mass Spectrometry

H Coffee Resprout Height

HPLC High Performance Liquid Chromatograph

IC Intensive Conventional

ICP-MS Inductively Coupled Plasma Mass Spectrometry

IO Intensive Organic

LC-MS	Liquid Chromatograph Mass Spectrometry
LO	Low Organic
LT	Legume Timber Shade Tree System
MC	Moderate Conventional
NLT	Non-legume Timber Shade Tree System
PB	Productive Branches
$S.E._D$	Standard Error of the Contrast Difference
S	Shaded System
SDS	Sodium Dodecyl Sulfate
Ser.	Service Shade Tree System
SIR	Selected Ion Recording
STD	Standard Mode
T	*Terminalia Amazonia*
TB	Total Branches
Tim.	Timber Shade Tree System

Chapter 1 Introduction

1.1 Coffee production

Coffee is one of the most popular beverages around the world. The main coffee producer worldwide is Brazil 30% (Perrone et al., 2008), followed by Africa (20.4%) and Asia (18.5%); however, consumers in Europe are the main coffee markets (Clarke, 1985). Coffee production generates over US$90 billion each year, making it one of the most traded agricultural products worldwide (DaMatta et al., 2007). In addition, coffee production plays an essential role in economic development of many tropical countries. Although world coffee production shows large fluctuations annually, it is generally increased by around 14% over the past decades (International Coffee Organization, ICO). The tremendous annual variation in coffee production, which is mainly caused by the recurring abiotic calamities such as frosts and droughts, has a dominating effect on fluctuations in world coffee supplies and market prices (Van der Vossen, 2001). There are two main kinds of coffee plants harvested worldwide: Coffea Arabica and Coffea Robusta (C. canephora), currently coffees derived from the Coffea Arabica bean are perceived as better or more flavorsome (Briandet, 1996). Coffea Arabica and Coffea Robusta share about 70% and 30% of the global coffee market. Vietnam and Indonesia are the two main countries where the robusta coffee is produced (Clarke and Vitzthum, 2008).

1.2 Coffee organic and agro-forestry systems

Coffee is a major export product for developing countries and provides livelihoods for millions of people, constituting a mayor income for many countries in South or Central America, Africa and South East Asia (DaMatta et al., 2007). Nonetheless, production methods are still highly conflicted between environmental impacts and productive needs.

Conventional treatment under full sun conditions has been improved over agro-forestry and/or organic managements, due to the higher coffee yield, at least under optimal growing conditions (Beer et al., 1997; DaMatta et al., 2007; Haggar et al., 2011). The increase of productivity has been achieved by using a large number of external agrochemical inputs and shortcutting ecological cycles (Beer et al., 1997; Haggar et al., 2011) which contributes to environmental pollution, degradation of soils (Lyngbaek and Muschler, 2001; DaMatta, 2004) and health hazards (Nygern, 2012). The high annual cost caused by these high external inputs cannot be reduced easily, which results in vulnerability of coffee farmers to the unstable international coffee market (Haggar et al., 2011). Nevertheless, environment, health and demand for sustainable agricultural products like organic coffee have led to 30 % of coffee production being sustainably certified (Potts et al., 2014). Moreover, organic coffee production and certification were found to reduce the vulnerability of coffee farmers income to seasonal shifts in price and raw material costs (Bacon, 2005), especially as farmers experimented with organic and shaded coffee production methods (Bacon, 2005; Haggar et al., 2011). In general, organically grown coffee was reported to yield lower than conventional on coffee farms in

Costa Rica, but a subgroup of farms in Costa Rica showed a similar or even higher productivity under the organic management when compared to the conventional management (Lyngbaek et al., 2001).

Although the coffee yield is significantly higher under the full sun system, agro-forestry systems have been suggested to have numerous benefits including protection of soil and water resources (Beer et al., 1997), reduced erosion and nitrogen leaching (DaMatta, 2004; Tully et al., 2012), buffering of climate extremes (Lin, 2007), less microclimatic variation (de Carvalho Gomes et al., 2016), higher carbon storage as well as higher local biodiversity (Tscharntke et al., 2011) and enhanced resource capture, such as light (Taugourdeau et al., 2014). Furthermore, legume shade tree species might replenish for the lower external inputs (Nygren et al., 2012; Tully et al., 2012). Under the sub-optimal growing conditions, the yield of shaded coffee exceeded those coffee grown under full sun conditions and shaded coffee had a lower yield bienniality (DaMatta, 2004; Vaast et al., 2005). Nonetheless, there is a serious disadvantage in shaded coffee system. It is the competition for growth resources such as light, water and nutrients between coffee plants and shade trees (Beer et al., 1997), which may reduce the coffee yield. In contrast to yield, vegetative growth is often similar or even higher under shade (Morais et al., 2003; Vaast et al., 2005) which demonstrates different responses of vegetative and reproductive coffee development to shade. The effects of the complex interactions between the tree and coffee component and management practices on the ecophysiology of coffee have been studied such as the number of nodes (Campanha et al., 2004), lateral growth (Beer et al., 1997; Campanha et al., 2004), height and diameter development (Morais et al., 2003) and their

relationships to coffee yield (Carvalho et al., 2011). Extensive long-term data on morphological coffee characteristics and relationships to yield, as well as proxies for yield would be extremely useful.

In the search for a more ecologically and economically sustainable coffee production the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) and local partners in Costa Rica established a coffee long-term experiment in 2000, using both conventional and organic managements of different intensities in plantations under full sun and under the shade of two legume and one timber tree species. The aim was to determine what levels of shade and which species characteristics were beneficial for different types and levels of agronomic inputs.

1.3 Coffee chemistry

Green coffee beans contain many nutriments such as carbohydrates, proteins, lipids, minerals, however, the amounts and varieties of these nutrients differ among the coffee beans with different field managements (Valkila, 2009). In addition, numerous physical attributes of green coffee beans such as density, bulk density and moisture content also impacted by the original and growth conditions in the field (Dutra et al., 2001).

In terms of the physical attributes of green bean such as green bean density, bulk density and moisture content, they also play a crucial role in coffee processing, storage, package and transportation (Dutra et al., 2001, Franca et al., 2005, Ismail et al., 2013). At the same time, these physical attributes of green coffee beans were determined by the original cultivation conditions of the coffee plant and the climate conditions in the field (DaMatta et al., 2007). During green coffee bean storage and transportation, moisture content, which is an essential part in determining the coffee storage stability against deterioration (Clarke, 1985), fluctuates due to the variation of relative humidity and temperature in circumstance. The change of moisture content leads to the variation of green bean mass (Amin et al., 2004; Pradhan et al., 2008). In addition, Ismail et al. (2013) stated that the coffee bean volume increased with increasing storage time. Thus, green coffee bean true density and bulk density also change during storage and transportation period. High density coffee is normally deemed to be more valuable in the international trade, as it outweighs and /or takes up less space than low density coffee bean (Kornman, 2016).

The minerals in food are crucial to human nutrition and health; minerals

contribute to the growth and formation of bones, synthesis of vitamins, enzymes and hormones, as well as for the healthy functioning of the nervous system, blood circulation and cellular integrity, if kept at required levels (McDowell, 1992). These essential minerals are normally classified into macro and microelements (Nabrzyski, 2007). Mineral substances have been found in coffee green and roasted beans (Oliveira et al., 2012; Grembecka et al., 2007). The main minerals presented in coffee are potassium (K), magnesium (Mg). phosphorus (P), Sulphur (S), calcium (Ca), sodium (Na), iron (Fe) and some trace elements such as copper (Cu), manganese (Mn), molybdenum (Mo) and zinc (Zn) (Arnaud, 1988). Coffee intake has been regarded as a good source of potassium, magnesium and manganese in the diet (Arnaud, 1988). Mineral content of coffee can be used to authenticate the geotype of the coffee samples as they reflect the soil type and environmental growing conditions (Martín et al., 1998). In addition, mineral contents of green coffee beans are also affected by the species and processing method (Arnaud, 1988). During the roasting process, minerals are significantly more stable than other chemical substances (Anderson and Simth, 2002; Grembecka et al., 2007), while it has been reported in some researches that minerals are considered as a catalyst in flavour formation during roasting (Arnaud, 1988).

The lipid content of green arabica coffee is about 15-17% on dry basis, while robusta coffee has a lipid content of around 10% (Speer and Kölling-Speer, 2006; Farah, 2012). Most of the lipid (coffee oil) is present in the endosperm of the coffee bean with a small quantity found in the outer layer of the coffee bean (coffee wax) (Wilson et al., 1997). These lipids are composed of triglycerides (75%), free and esterified diterpene alcohols (19%), free and esterified sterols

(5%) and very small amounts (~1%) of other substances, such as tocopherols, phosphatides and tryptamine derivatives (Maier, 1981). The fatty acid content in coffee has been well researched and has revealed that the majority of fatty acids present in coffee are esterified with glycerol into triacylglycerols, around 20% are esterified with diterpenes, and some become esterified with sterol (Speer, 2006). The lipid types and content in green coffee beans are determined by the coffee plant origin and external growth conditions such as light intensity and fertilizer level (Speer and Kölling-Speer, 2001; Ohlrogge and Browse, 1995). Presence of free fatty acid (FFA) in coffee has been reported by many researchers (Carisano and Gariboldi, 1964; Wajda and Walczyk, 1978). Eleven different fatty acids have been detected in both arabica and robusta coffee; these include the main fatty acids linoleic acid (C18:2) and palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), arachidic acid (C20:0), behenic acid (C22:0), palmitoleic acid (16:1) and cosenoic acid (20:1). Small concentrations of myristic acid (C14:0), linolenic acid (C18:3) and lignoceric acid (C24:0) have been found (Speer and Kölling-Speer, 1985). In addition, fatty acids esterify with glycerol to generate triacylglycerol stored in the green coffee beans to supply the energy and nutrients for the next generation (Ohlrogge and Browse, 1995). During the roasting process, there are no significant changes in fatty acid composition (Folstar, 1976).

The carbohydrates, which play a crucial role in coffee roasting, constitute about half of the dry weight basis of green coffee bean (Bradbury, 1990). The main low molecular weight carbohydrate present in green coffee bean is sucrose accounting for about 8% of bean dry weight, and also, some trace amounts of monosaccharides such as fructose, glucose, mannose and arabinose are

reported in the green coffee bean (Silwar and Lüllman, 1988). In addition, a few polyalcohols and diterpene glycosides are found in the green coffee beans. There are three main polysaccharides including arabinogalactan, mannan (and/or galactomannan) and cellulose present in the green coffee bean, although the presence of other monosaccharides such as xylose and rhamnose in acidic hydrolysates of green coffee bean provides the evidence for other polymers (Bradbury and Halliday, 1990). During the coffee roasting progress, carbohydrates especially sucrose are degraded, dehydrated and interacted with amino acids or protein to generate a large number of volatile compounds (Tressl et al., 1998; Ginz et al., 2000; Bradbury, 1990), which contributes to the formation of organoleptic properties of roasted coffee beans including flavour, aroma and colour (Bottazzi, 2012). The carbohydrates types and contents vary with the species and the external cultivation and environment of coffee plant (Boardman, 1977; Robarts and Zohary, 1987; Silwar and Lüllman, 1988).

Proteins have been shown to contribute to flavour and colour formation during the roasting process, however, compared with free fatty acids (FFA), the reactivity of amino acids combined with protein is lower during roasting due to rapid degradation (Homma, 2001; Liu and Kitts, 2011). Recent research has revealed that protein or peptides might contribute to the formation of aroma, bitter taste and metal-chelating compounds in coffee brews (Grosch, 2001; Montavon et al., 2003). Research into the composition of proteins in coffee has revealed that coffee consists of 15% albumin and 85% globulin (Ludwig et al., 1995) and its content was around 10-16% of the green coffee bean dry weight (Macrae, 1985; Rogers et al., 1997; Farah, 2012). During the roasting process, the proteins and amino acids of green coffee beans, which are precursors of

many important aroma compounds found in roasted coffee, play an essential role in the formation of flavour and colour as a result of pyrolytic and Maillard reactions (Homma, 2001; Moreira et al., 2012). Therefore, the crude protein content of green coffee beans may be used as a reference when deciding the best roasting conditions (Homma, 2001).

Caffeine, which is the most important purine alkaloid with a bitter taste present in the green coffee bean, has been investigated for many years (Ashihara and Crozier, 2001). The anhydrous caffeine is stable in contact with aqueous solution above 52°C, while only the hydrate is stable when temperature is below 52°C (Macrae, 1985). The caffeine content of green coffee bean varies widely in the different species and the robusta coffees have a higher caffeine content with a mean value of 2.2% by dry basis than the arabicas with a mean value of caffeine content around 1.2% by dry basis (Macrae, 1985). In addition, the effect of environment and cultivation on the caffeine content of green coffee bean has been reported by some researchers (Macrae, 1985). Caffeine is also very stable during the coffee bean roasting process, although the roasting temperature is well in the excess of the sublimation point of caffeine, as the sublimation point of caffeine increases during roasting due to pressure build-up within the bean and a low diffusion rate of vapour through its outer layers (Macrae, 1985). Ogita et al. (2004) stated that caffeine was used as a stimulant in the human's central nervous system.

In conclusion, many physical and chemical attributes of green coffee bean were introduced and almost all of these characteristics were related to the species, the external environment and the agricultural management of coffee plant,

nevertheless, a few publications connected the attributes of green coffee bean with the agricultural treatments were reported in the last few decades. As a result, it is necessary for coffee scientists to explore the relationships between the species and the growth conditions and the physical and chemical attributes of green coffee bean.

1.4 Aroma generation in coffee

Coffee is widely appreciated and consumed due to its pleasant flavour generated during the roasting process. In addition, expert coffee tasters assess the quality of coffee, to a greater extent, by its aroma and taste, and the highest quality coffee beans can obtain a considerable price premium (Dart and Nursten, 1985). A large number of investigations, therefore, have been carried out to evaluate the types and quantities of volatile compounds as well as its formation principals in the roasted coffee beans. Most volatile compounds formed during roasting are derived from a range of chemical precursors in the green coffee beans, these include sugars, amino acids, organic acids and phenolic compounds, and these react through many complex reactions including the Maillard reaction, degradation of sugars and lipid oxidation. (Tressl, 1989; Holscher and Steinhart, 1994; Ho et al, 1993; Reineccius, 1995). Nine specific pathways to generate the volatile compounds during roasting of coffee have been outlined by Dart and Nursten in 1985. There were the Maillard reaction, Strecker degradation, trigonelline degradation, phenolic acids degradation, lipid degradation, sugar degradation, breakdown of sulphur amino acid, breakdown of hydroxyl amino acids and proline and hydroxyproline degradation. Moreover, the dominating volatile compounds, according to their functional groups, have been divided into ten classes by Dart and Nursten in 1985. They included sulphur compounds, pyrazines, pyridines, pyrroles, oxazoles, furans, aldehydes, ketones, phenols and other heterocyclic compounds. Many factors, including coffee bean species, the external environment and soil conditions, storage of both green and roasted coffee beans, the roasting conditions and the roasting equipment, will affect the final

composition of volatile compounds in the roasted coffee beans and the coffee drinks after brewing (Dart and Nursten, 1985).

Coffee roasting plays an essential role in the formation of organoleptic properties including flavour, aroma and colour. During the roasting process, moisture content decreases and many chemical reactions occur, along with crucial variations in terms of flavour, colour, volume, weight, bean pop, pH, density and presence of volatile compounds (Bottazzi, 2012). The roasting process can be divided into two main steps: drying and roasting. In the first stage, bean temperature is below 160°C and the water content descends from around 8-12% downwards (Gloess, 2014). In the second stage, pyrolytic reactions occur at 190°C leading to oxidation, reduction, hydrolysis, polymerisation, decarboxylation and many other chemical reactions, which causes the formation of coffee flavour, aroma and colour (Singh et al., 1997). In addition, roasting also generates CO_2, some CO_2 escapes and some is kept in the cells of the roasted beans (Schwartzberg, 2002). After the second step, the beans have to be quickly cooled to stop any further reactions, either by water or air and to avoid an excessive roast, which is perceived to damage product quality (Gloess, 2014).

During the roasting progress, a large number of volatile compounds are produced due to Maillard reactions, Strecker degradation, pyrolysis, and other chemical reactions (Schenker et al., 2002). Whilst more than 800 different volatile compounds from a wide range of chemical classes (including hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, pyrazines, pyrroles, pyridines, sulphur compounds, furanes, phenols and so on) have been

identified in roasted coffee (Nijssen et al, 1996) their relative importance in the flavour of the finished coffee varies. In recent years, research has highlighted the sensory relevance of these volatile compounds and has identified the key odourants which are mono- and dicarbonyl, furanones, alkylpyrazines and phenols in coffee beans and brewed coffee beverages (Czerny et al., 1999, Grosch, 2001).

Gas chromatography-mass spectrometry (GC-MS) has been widely used in the analysis of volatile compounds in coffee in the last few decades due to its sensitivity and operability. It can separate and identify a complex mixture of aromas in one operation as well as quantify the volatiles at extremely low concentrations (Dart and Nursten, 1985).

Numerous researches have been carried to identify the volatile compounds of coffee, explore the formation mechanisms of these pleasant aromas and investigate the relationship between the composition of green coffee beans and aroma generation of roasted coffee beans (Aeschbacher et al., 1989; Bade-Wenger et al., 1993; Amrani-Hemaimiet al., 1995; Blank, 1998; Czerny and Grosch, 2000), but only a limited number of studies have actually connected these to the growth conditions, levels and types of fertilizer, the species of shade trees. As a result, it is necessary for coffee scientists to explore the interactions and detail the specific effects of fertilizer levels and shade types on aroma generation of roasted coffee beans.

1.5 Experimental aims and hypotheses

The aroma of coffee is generated principally through Maillard Chemistry and lipid oxidation; it can therefore be assumed that different sunlight intensities and nitrogen, phosphorous and potassium sources will result in significantly different sugar, lipid and amino acid profiles; which will ultimately result in significant changes to Maillard Chemistry and lipid oxidation pathways. The overarching aim of this project was to correlate agro-forestry practices of coffee with macro and micro nutrient deposition of green coffee bean and the resultant aroma of roasted coffee. At the same time, the effect of different fertilizer levels and shade types on coffee yield and morphology of coffee trees was investigated. This thesis examines three main parts: 1) the impact of agro-forestry systems on coffee tree yield, pruning intensity, morphology including coffee tree height, diameter, total branches and productive branches and shade cover; 2) the impact of agro-forestry systems on the physical and chemical properties of green coffee bean; 3) the impact of physical and chemical properties of green coffee bean on the aroma generation of roasted coffee bean, and further to investigate the impact of agro-forestry systems on the aroma generation of roasted coffee bean. The specific hypotheses to be tested in this research project were:

Agro-forestry systems affect the coffee tree yield and morphology (Chapter 3)

1. Different management methods including fertilizer level of soil, weed control and pruning density affect coffee tree yield and morphology due to different intensities of nutrient intake and growth conditions.

2. Different shade trees affect coffee tree yield and morphology due to different sunlight intensities, competition environment and nitrogen fixation.

Agro-forestry systems affect the physical and chemical properties of green coffee bean (Chapter 4)

3. Different treatments including management methods and shade trees affect the physical and chemical properties of green coffee bean due to different intensities of nutrient intake and sunlight, growth conditions and nitrogen fixation of shade tree.

Agro-forestry systems affect the aroma generation of roasted coffee bean (Chapter 5)

4. Different treatments including management methods and shade trees affect the aroma generation of roasted coffee bean, as different intensities of nutrient intake and sunlight, growth conditions and nitrogen fixation of shade tree will result in significantly different sugar, lipid and amino acid profiles, which will ultimately lead to significant changes to Maillard Chemistry and lipid oxidation pathways, and further to affect aroma generation.

This thesis examines the relationships between different coffee agro-forestry systems and aroma generation of roasted coffee. In the following chapter of the thesis, Chapter 2, illustrates all methodologies. Chapter 3 investigates the impact of agro-forestry systems on coffee tree yield, pruning intensity, morphology and shade cover. Chapter 4 explores the impact of agro-forestry

systems on the physical and chemical properties of green coffee bean. The impact of physical and chemical properties of green coffee bean on the aroma generation of roasted coffee bean, and the impact of agro-forestry systems on the aroma generation of roasted coffee bean are examined in Chapter 5. Finally, the comprehensive discussions and conclusions and possible future work are discussed in Chapter 6 and 7.

Chapter 2 Materials and methods

2.1 Agro-forestry methodologies

2.1.1 Experimental design

The experiment was established in 2000 at CATIE (Tropical Agricultural Research and Higher Education Center), Turrialba, Costa Rica (9°53’44’’ N, 83°40’7’’ W, CATIE, Turrialba, Costa Rica), which is defined as a low altitude (600 m above sea level), wet coffee zone without a marked dry season. Average annual rainfall, temperature, relative humidity and solar radiation was 2,915 mm/year, 22°C, 90.2% and 15.9 MJ/m^2/year (2000-2013).

Twenty agroforestry systems with different shade types and managements consisting of an incomplete randomized block-design with shade tree as main effect and subplots represented by management were set up (Table 2.1). For each system, three replicates were established. Shade type (initially 417 trees per ha^{-1} (6 x 4m^2 spacing)) consisted of timber and service tree species with contrasting characteristics (Table 2.2). Fig. 2.1 showed the photos of three shade trees. Trees were progressively thinned to maintain a reasonable shade environment for coffee production (Table 2.3).

Table 2.1 Agroforestry systems with main plot (Shade type) and subplot (Management) treatments

Shade types*	E	T	C	C+T	E+T	C+E	Full Sun
Managements**	IC	IC				IC	IC
	MC	MC	MC	MC	MC	MC	MC
	IO	IO	IO	IO	IO	IO	
	LO	LO				LO	

*E: *Erythrina poepiggiana*, C: *Chloroleucon eurycyclum*, T: *Terminalia amazonia*;
**IC: Intensive conventional, MC: Moderate conventional, IO: Intensive organic, LO: Low organic; (n=3)

Table 2.2 Characteristics of shade trees (Haggar et al., 2011)

Species	Phenology	Canopy	N-fixer	Use
Erythrina poepiggiana (E)	Evergreen	Low compact	Yes	Service
Chloroleucon eurycyclum (C)	Deciduous *	High spreading	Yes	Timber
Terminalia amazonia (T)	Deciduous *	High compact	No	Timber

* Deciduous for about 20-30 days per year



Erythrina poepiggiana



Chloroleucon eurycyclum



Terminalia Amazonia

Fig. 2.1 Photos of three shade trees

Table 2.3 Mean shade tree density after thinning

Agroforestry system		Tree density per ha^{-1}		
System	Tree species	2008	2011	2013
Monocultures				
E	E	360	285	241
C	C	381	154	65
T	T	317	167	73
Polycultures				
C+E	C	183	100	45
	E	181	134	115
C+T	C	166	77	39
	T	170	77	34
E+T	E	147	143	109
	T	158	81	34

Intensive conventional (IC) *Erythrina* trees were biannually pollarded to a 1.8-2.0 m main trunk. Whilst this is normal practice in Costa Rica, Muschler (2001) found that coffee quality benefited from increased *Erythrina* shade levels, therefore, for all the other treatments with *Erythrina*, trees were pollarded to 4 m leaving three branches for partial shade. Temporary shade was planted in the form of *Ricinus* in organic treatments. This took place one year after the coffee plants, to improve coffee plant survival and impede weed growth. Lower branches of the timber trees were pruned annually (year 1-7) to improve stem quality. In all pruning scenarios, pruning residuals from coffee trees and shade trees were left on the ground (trunks were removed). Management consisted of fertilization, weed, disease and pest control, detailed in Table 2.4.

Table 2.4 Mean input levels of fertilizers (Kg $ha^{-1}year^{-1}$) and weed/disease control since 2006, adapted from Haggar et al. (2011) and Noponen et al. (2012)

Management	Fertilization N:P:K **	Weed control	Disease/Pest control
IC	287:20:150	6* Herbicides	3-4* Fungicides/ Insecticides
MC	150:10:75	5 Herbicides 4 Manual	1-4 Fungicides/ Insecticides as required
IO	248:205:326	4 Manual	Organic substances as required
LO	66:2:44	4 Manual	No

* Number of treatments applied per year.

** Fertilization levels (Kg $ha^{-1}year^{-1}$) are 7 year means (2003-2009), from the second to forth year LO systems received the same fertilization as IO ones, due to the site limitations that did not allow organic coffee to establish effectively with lower inputs. IO fertilization: chicken manure 10 t $ha^{-1}year^{-1}$and K-Mag (fertilizer)100kg $ha^{-1}year^{-1}$; LO fertilization: Coffee pulp 5 t $ha^{-1}year^{-1}$

Coffea Arabica L. var. *Caturra,* was planted at 5000 holes ha^{-1} with dead plants replaced each year. Two plants per planting hole were planted (local practice) but were treated as one plant in every analysis. The distance between rows and

holes were 2 m and 1 m. Sub-plots were 500-600 m^2 of which the central 225-300 m^2 was studied (100 coffee plants and 24 shade trees).

Coffee plants were manually pruned from 2004 leaving 1-4 resprouts per stump, according to the productive potential of each coffee resprout in the next harvest. Every coffee planting hole thus comprised 1 - 2 stumps and a total of 1 – 4 resprouts per stump. Fig. 2.2 showed the map of three blocks (three replicates) in the field.



Fig. 2.2 Map of three blocks (three replicates) in the field

2.1.2 Annual coffee yield and biennial production

Coffee yield was measured by weighing fresh coffee cherries harvested per plot. The data was collected by two employed local farmers: Luis Romero and Luis Araya. The author SU XU weighed the fresh coffee cherries in CATIE, Costa Rica, in September 2014. The data was analysed by SU XU and Florian Schnabel in CATIE, Costa Rica. Bienniality index (BI) of coffee yield, an index for the difference between two successive years, (Cilas et al., 2011) with modifications.

$$BI = (|y_2 - y_1| + |y_3 - y_2| + \ldots + |y_n - y_{n-1}|) / (N-1)$$

Where: y_i coffee yield (y) for year i; N Total number of years

2.1.3 Coffee pruning intensity

In each treatment plot, the cumulative percentage of totally and partially (some resprouts only) pruned coffee plants was recorded annually (2004-2014). The data was collected by two employed local farmers: Luis Romero and Luis Araya and Elias de Melo Virginio Filho who was responsible for the field experiment. The data was analysed by SU XU and Florian Schnabel in CATIE, Costa Rica.

2.1.4 Coffee plant morphology

Coffee resprout height (H) (from the soil surface to the top), diameter (D) and the total number of branches (TB) were measured in 2002 and 2014. In 2014, the number of productive branches (PB) (>60% living tissue) was also measured. Diameter was measured 5 cm above the intersection with the main

stump or 10 cm above the ground if there was no pruning.

Twenty-four coffee plants, equally spaced, were selected in each plot in 2002, while twenty-six coffee plants were measured in 2014. The highest resprouts were measured up to a maximum of 4 resprouts for each planting hole. The highest resprout was defined as the dominant one, the rest of the resprouts were regarded as secondary ones. The data for dominant resprout and the average of secondary resprouts were recorded separately per plot and finally averaged to create one single morphological variable. Fig. 2.3 and 2.4 showed the schematic diagrams for selecting the coffee plants used to collect data in each plot and measuring the height and diameter of coffee plant.

The data was collected by previous students in CATIE, Costa Rica, in 2002, while the data was collected by SU XU and Florian Schnabel in CATIE, Costa Rica, in 2014. All data was analysed by SU XU and Florian Schnabel in CATIE, Costa Rica.

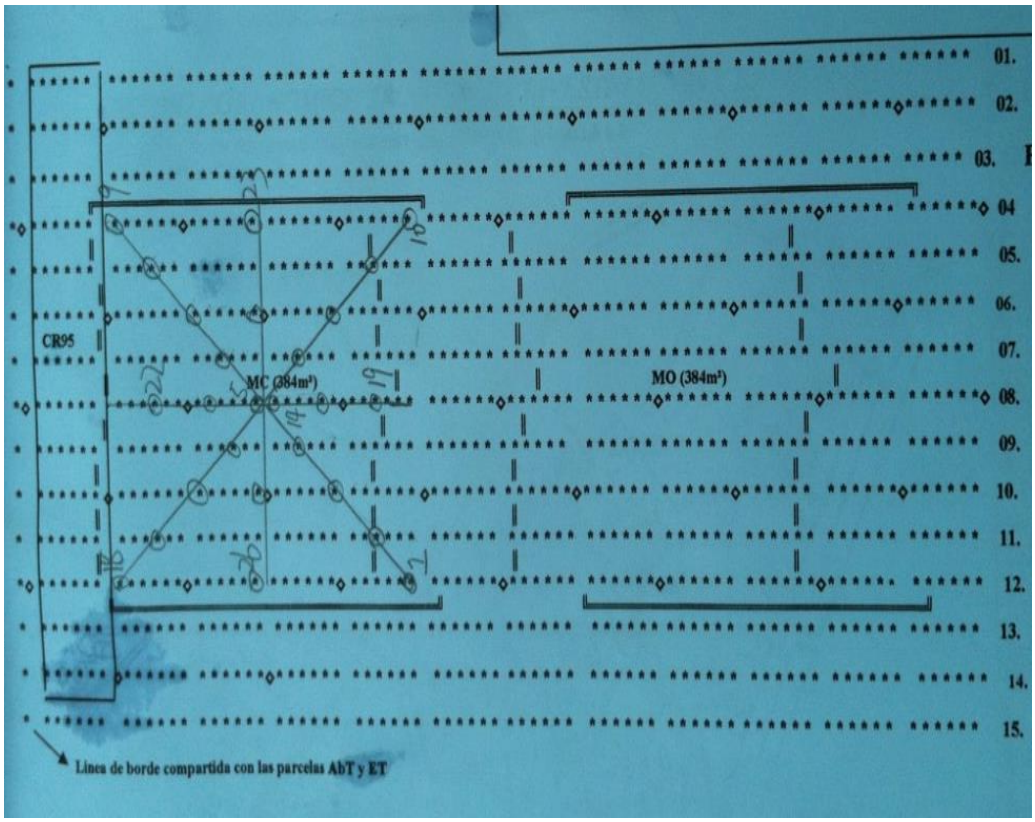


Fig. 2.3 The schematic diagram for selecting the coffee plants used to collect data in each plot



Fig. 2.4 The schematic diagrams for measuring the height and diameter of coffee plant

2.1.5 Shade cover

Absolute and average shade cover (%) over seven months per plot was estimated monthly (January 2014 – August 2014, without May) using a densiometer, following Lemmon (1956). Four measurement points equally spaced were selected along with the East-West diagonal of each plot. Shade cover was recorded in each detecting point from four directions (North, South, West and East). Fig. 2.5 showed the schematic diagrams for the densiometer and measuring the shade cover of shade trees. SU XU collected the shade cover data in July and August 2014 in CATIE, Costa Rica and the rest data was measured by local students in CATIE, Costa Rica. All data was analysed by SU XU and Florian Schnabel in CATIE, Costa Rica.



Fig. 2.5 The schematic diagrams for the densiometer and measuring the shade cover of shade trees

2.2 Physical and chemical analysis methodologies of green and roasted coffee beans

The data of physical and chemical properties of green coffee beans harvested from Costa Rica in 2013 and 2014 was collected and analysed by SU XU in the University of Nottingham, UK.

2.2.1 Coffee roasting

All green coffee beans harvested in different agro-forestry systems from CATIE, Costa Rica in both 2013 and 2014 were sent to Edgehill Coffee Company (Warwick, UK) for roasting. The coffee roaster (Roastilino, Fracino) with a small fluidised bed and a nominal capacity of about 200g of green coffee beans was used and 50g of green coffee beans were prepared and roasted. The air outlet temperature and roasting time were 166°C and 1 min 50 s, respectively. All coffee beans were taken past first crack and roasted to medium roasting. Fig 2.6 showed the photo of coffee roaster (Roastilino, Fracino). After roasting, 30 g of different roasted coffee beans were ground into powders using a mini chopper (CH180 mini chopper, 300watt, Kenwood) and a 710 μm sieve was used to obtain even coffee powder samples. All coffee beans and powders were stored at -20°C.



Fig. 2.6 Photo of coffee roaster (Roastilino, Fracino)

2.2.2 Measurement of roasting loss

The roasting loss was measured by the Edgehill Coffee Company. Green and roasted coffee beans were weighed using a balance (0.01g, PFB 1200-2, KERN Ltd.) before and after roasting and their weights recorded (W_1 for green beans, W_2 for roasted beans). Thus, roasting loss (g) = W_1(g)-W_2(g).

2.2.3 Measurement of moisture content

All coffee samples were weighed without containers and the weight recorded W_1 (g). All coffee samples were weighed together with their containers using balance (0.01g, PFB 1200-2, KERN Ltd.) and their total weight recorded W_2 (g). Samples were placed into a drying oven at 105°C (GenLab Ltd.). After 24

h, coffee samples were weighed together with their containers again and their weight recorded W_3 (g) (Baggenstoss et al., 2007). Thus:

$$Moisture\ content\ \ (g/kg) = \frac{W_2\ (g) - W_3\ (g)}{W_1\ (g)} \times 1000$$

2.2.4 Measurement of colour

The colorimeter (ColorQuest XE, HunterLab Inc.) was used to measure the colour of the coffee bean (Baggenstoss et al., 2007). The colorimeter was calibrated using the white plate and packed coffee powder was added to a transparent preservative film and then placed on the detection port to obtain data. Results were calculated by the equipment using the Hunter Lab colour scale. In this scale, L ranges from 0 (black) to 100 (white), a indicates degree of greenness (for negative a values) and degree of redness (for positive a results), b axis also ranges from negative to positive values indicating, respectively, degree of blueness to yellowness.

2.2.5 Measurement of green coffee bean density

The assumption was that each green coffee bean could be taken as half a triaxial ellipsoid (Dutra et al., 2001). The length (major), width (minor) and thickness of each green coffee bean were measured using a Vernier-caliper (Accuracy: ± 0.02mm, Carbon Fibre Composites Digital Caliper, Fisher Scientific) and recorded as a, b and c, respectively (Fig. 2.7). Thus, the volume of each green coffee bean could be calculated using the ellipsoid volume formula as the following:

$$The\ volume\ of\ individual\ bean\ (mm^3) = \frac{1}{2} \times \frac{4}{3}\pi \frac{a}{2}\frac{b}{2}c = \frac{\pi abc}{6}$$

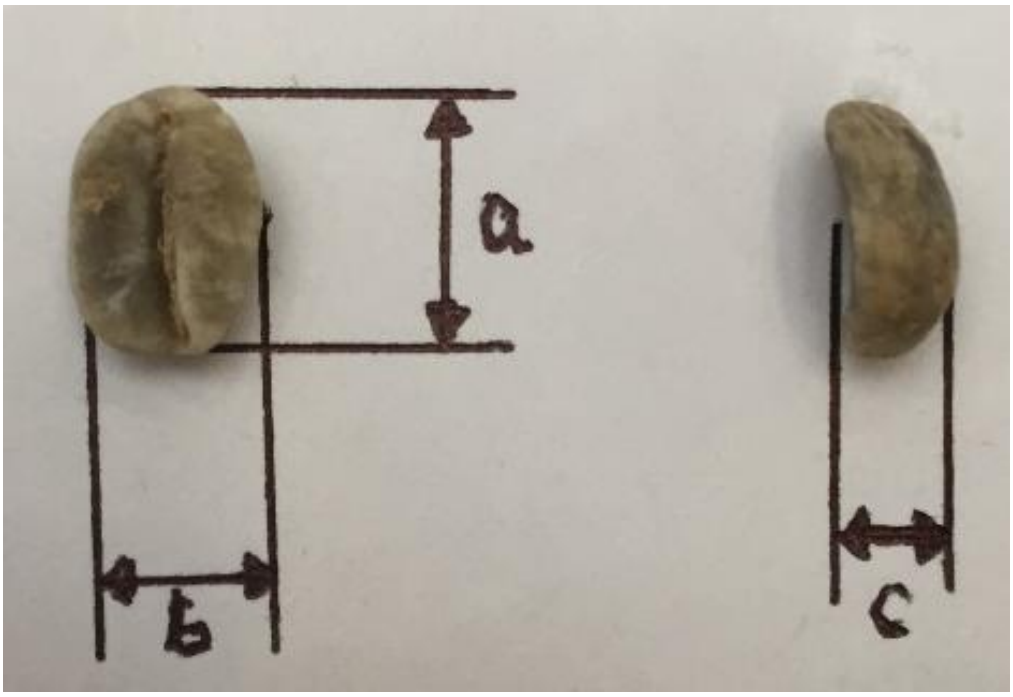


Fig. 2.7 Coffee bean dimensions

10 green coffee beans (3 replicates) were randomly selected from each plot and their length, width and thickness were measured (Franca et al., 2005). After that, the volumes of each green coffee bean were calculated, and then, the average volume (V_a (mm^3)) of the 10 green coffee beans was calculated. The total weight (W_1 (g)) of 10 green coffee beans was measured using a balance (0.01g, PFB 1200-2, KERN Ltd.). Thus, the average density of individual bean could be calculated as below:

$$The\ average\ density\ of\ individual\ bean\ (kg/m^3) = \frac{W_1\ (kg)}{V_a\ (m^3)}$$

2.2.6 Measurement of bulk density

Around 20g of green coffee beans (3 replicates) were weighed using a balance (0.01g, PFB 1200-2, KERN Ltd., Fareham, UK). After that, the green coffee beans were put in a volumetric flask and the volume was recorded (Franca et al., 2005). Thus, the average bulk density of green coffee beans could be calculated.

2.2.7 Measurement of mineral content

The acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) were used to prepare to the sample and measure the mineral content of green coffee beans (Chilimba et al., 2012).

For acid digestion, 0.2 g of coffee powder was placed into plastic tubes provided by Microwave Digestion System (Microwave Digestion System: Multiwave Go, AutonPaar). 6 mL of 70% nitric acid (Trace Analysis Grade, Fisher, UK) was added into the plastic tubes and then placed into the microwave digestion system for 40 min with a heating program: the system temperature increased from 0°C to 160°C over 15 min, held at 160°C for 15 min and then cooled to room temperature for 10 min. After incubation, samples were transferred to glass bottles. 4 mL, 5 mL and 5 mL of distilled water were used sequentially to clean the plastic tubes and then transferred to glass bottles, separately. Finally, 2 mL of samples were transferred to inductively coupled plasma (ICP) tubes and 8 mL of distilled water was added.

For inductively coupled plasma mass spectrometry (ICP-MS) analysis, multi-element analysis of diluted solutions was undertaken by ICP-MS (Thermo-

Fisher iCAP-Q model, Thermo Fisher Scientific, Bremen, Germany). The instrument was run by employing three operational modes, including (i) a collision-cell (Q cell) using helium (He) with kinetic energy discrimination (He-cell) to remove polyatomic interferences, (ii) standard mode (STD) in which the collision cell is evacuated and (iii) hydrogen mode (H_2-cell) in which H_2 gas is used as the cell gas. Samples were introduced via an autosampler (Cetac ASX-520) incorporating an ASXpress rapid uptake module through a PEEK nebulizer (Burgener Mira Mist). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L^{-1}), Rh (10 µg L^{-1}) and Ir (5 µg L^{-1}) in 2% trace analysis grade (Fisher Scientific, UK) HNO_3. External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) included Ag, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, U, V and Zn, in the range 0 – 100 µg/L (0, 20, 40, 100 µg/L). A bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, France) was used to create Ca, Mg, Na and K standards in the range 0-30 mg/L. Phosphorus, boron and sulphur calibration utilized in-house standard solutions (KH_2PO_4, K_2SO_4 and H_3BO_3). In-sample switching was used to measure B and P in STD mode, Se in H_2-cell mode and all other elements in He-cell mode. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required.

2.2.8 Measurement of total lipid content and fatty acid

2.2.8.1 Lipid extraction:

The Bligh and Dyer method (1959) was used to extract lipids from coffee. 0.1 g of coffee powders (wet basis) was placed into a 15 mL centrifuge tube. 2 mL of chloroform: methanol (2:1) was added and vortexed for 1 min and then samples were centrifuged at 1600 g for 10 min at 4°C. After centrifugation, there were three phases presenting in the samples; the bottom phase was carefully removed and placed into a new glass vial. The above processes were repeated three times to extract lipids completely. The bottom phase was then exposed to N_2 to avoid lipid oxidation and stored, the lyophilized lipid samples were stored at -80°C with N_2 added into the storage bottles to protect the lipid.

2.2.8.2 Measurement of total lipid content

Around 0.1g of coffee powders (wet basis) was prepared and the weight was recorded W_1 (g) using an analytical balance (0.1mg, Sartorius 1712, Sartorius Ltd., Epsom, UK). An empty glass vial was weighed and the weight recorded W_2 (g). For each sample, the bottom phases of three extractions were combined into the glass vial and dried using N_2 gas. The weight of the glass vial containing the dried lipid extract was recorded W_3 (g) (Nantiyakul et al., 2013). The total lipid content by dry mass was calculated using the following formula:

$$The\ total\ lipid\ content\ (dry\ mass)(g/kg) = \frac{W_3(g) - W_2(g)}{W_1(g) \times (1 - MC)} \times 1000$$

Where MC was moisture content as a percentage.

2.2.8.3 Measurement of fatty acids

2.2.8.3.1 Preparation of internal standard solution

0.1 g of methyl pentadecanoate was weighed and dissolved in 10 mL chloroform.

2.2.8.3.2 Preparation of samples

2 mL of chloroform was added to the dried lipid extract and vortexed for 2 min. 1 mL of the mixture was filtered (0.45 µm, PTFE syringe filter, Whatman, Buckinghamshire, UK) and transferred to a new glass vial. 100 µL of internal standard was added and then 200 µL of trimethylsulfonium hydroxide solution was added for the conversion of fatty acids into fatty acid methyl esters (FAMEs). The mixture was incubated at room temperature for 10 min to ensure complete conversion. Finally, the mixture was placed into a gas chromatography (GC) vial for analysis (the method was modified from Nantiyakul et al., 2012).

2.2.8.3.3 Gas chromatography-mass spectrometry (GC-MS) analysis

A gas chromatograph (Trace GC Ultra, Thermo Scientific) equipped with an auto injection system (AS3000) and coupled to a quadruple mass spectrometer (DSQII Quadrupole GC-MS, scanning from 50 to 380 m/z, Thermo Scientific) was employed. 1 µL of sample was injected into a capillary column (Phenonenex Zebron ZB-FFAP, 30 m x 0.25 mm I.D, 0.25 µm film thickness) using a vaporisation injector in split mode (3.0 mm liner, 250°C, split flow 50ml/min). The oven temperature was initially held at 120°C for 1 min and increased to 250°C at a rate of 5°C/min, held for 2min. Retention time of each

peak was compared with FAME standards (Supelco37 Component FAME Mix). Identification was verified by comparing the mass spectrum with a standard library through Thermo Scientific Xcalibur software program. The peak area of each fatty acid was calculated using the Thermo Scientific Xcalibur software program. The content of fatty acid was calculated using the formula below (the method was modified from Nantiyakul et al., 2012):

$$Fatty\ acid\ concentration\ (dry\ mass)\ (mg/kg) = \frac{Peak\ area\ of\ fatty\ acid \times Amount\ of\ internal\ standard\ (mg)}{Peak\ area\ of\ internal\ standard \times Dry\ weight\ of\ coffee\ powder\ \ (kg)}$$

(Dry weight of coffee powder = wet weight of coffee powder × (1-moisture content))

2.2.9 Measurement of total protein content

A bicinchoninic acid (BCA) assay kit (23225/23227, Thermo Scientific) was used to measure the total protein content (Fisk et al., 2009).

0.1 g of coffee powders (wet basis) was weighed and 2 mL of chloroform was used to extract lipid with three replicates. After lipid extraction, the solid phase was dried at 60°C for 30 min and placed in a 15 mL centrifuge tube. After that, 1 mL of 2% sodium dodecyl sulfate (SDS) solution was added into the tube to eliminate interference from lipids and the tube was heated at 60°C in a water bath for 30 min. The sample was then vortexed for 1 min and centrifuged at 13,000 g for 3 min. And then, samples were filtered and diluted 100 times using 2% SDS.

According to the instructions of BCA protein assay kit, 0.1 mL of each

standard and sample replicate was added into test tubes. 2.0 mL of the working reagent containing 50 parts of BCA Reagent A (sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) and 1 part of BCA Reagent B (4% cupric sulfate) was added to each tube and mixed well. All tubes were covered and incubated at 37°C for 30 min, after incubation, tubes were cooled to room temperature. The absorbance of the samples was measured at 562 nm and subtracted the average 562 nm absorbance measurement of the Blank standard replicates. A standard curve was prepared by plotting the average blank-corrected 562 nm measurements for each bovin serum albumin (BSA) standard versus its concentration in µg/ml. Finally, the standard curve was used to determine the protein concentration of each sample

2.2.10 Measurement of sucrose and caffeine content

2.2.10.1 Standard preparation

The sucrose and caffeine mixture standard was prepared at a concentration of 10, 20, 40 and 50 mg/L.

2.2.10.2 Sample preparation

0.1 g of coffee powder was placed in a 50 ml centrifuge tube with 20 ml of boiling water and vortexed for 5 min. Samples then were centrifuged at 1600 g for 10 min at room temperature. After centrifugation, the liquid phase was transferred into a new glass vial. The above processes were repeated three times for extracting sucrose and caffeine completely. The mixture was cooled to room temperature and then filtered using a syringe filter (0.45 µm,

hydrophilic nylon syringe filter, Millipore Corporation). The final extract was diluted with water (1:1) prior to Liquid chromatography-mass spectrometry (LC/MS) analysis (the method was modified from Ky et al., 2000, McCusker et al., 2003 and Perrone et al., 2008).

2.2.10.3 Liquid chromatography-mass spectrometry (LC-MS) analysis

The LC equipment (1100 Series, Agilent) consisted of a degasser (G1322A, Agilent), a pump (G1312A, Agilent), an auto-sampler (G1313A, Agilent). This LC system was interfaced with a Quattro Ultima mass spectrometer (Micromass, UK Ltd.) fitted with an electrospray ion source.

The Kromasil 5 ODS (C18) column (250 × 3.20 mm, 5 µm, Phenomenex) was used to separate sucrose and caffeine and the column was maintained at room temperature. The mobile phase consisted of 0.3% aqueous formic acid (eluent A) and methanol (eluent B) which was delivered at a flow rate of 0.4 ml/min. Initially the solvent was 25% B. After the injection, this proportion was changed immediately to 60% B until the end of the run at 8 min. Between injections, the column was re-equilibrated with 25% B for 4 min.

The electrospray ionization source was operated in the negative mode (3.5KV) from 0.0 to 4.0 min to generate the formic acid adduct of sucrose $(M+HCOO)^-$ and in the positive mode (3.5KV) from 4.0 to 8.0 min to generate caffeine $(M+H)^+$ ions. Desolvation gas (N_2) flow rate was 500 L/h and desolvation temperature was 400°C. The mass spectrometer was operated in the selected ion recording (SIR) model to measure sucrose and caffeine. Identification of compounds was performed by comparing retention time and molecular weight of the respective standards. Quantification was achieved by comparing peak

areas of samples and standards using Masslynx software (version 4.0, Waters) (the method was modified from Ky et al., 2000 and McCusker et al., 2003).

2.2.11 Measurement of carbohydrate content

2.2.11.1 Standard preparation

The carbohydrate standard mixture including arabinose, galactose, glucose and mannose were prepared. The concentration of arabinose and glucose were 2.5, 5, 10 and 20 mg/L, while the concentration of galactose was 5, 10, 40 and 80 mg/L and the concentration of mannose was 5, 10, 20 and 40 mg/L.

2.2.11.2 Sample preparation

Saeman's hydrolysis (Saeman, 1945) was used to extract carbohydrates from green coffee beans. 30 mg of green coffee powder (wet basis) were placed into a round bottomed Pyrex tube and 1ml of 12M H_2S0_4 was added, sealed and the samples then were placed into a water bath at 37^{o}C for 60 min. The samples then were removed and 11 mL of demineralised water was added. The samples were then placed in a water bath at 100^{o}C for 120 min. After that, the samples were removed and placed in a cold water bath until they were cool enough to handle. 1mL of the sample was then placed into a 15 mL Falcon tube and 9 mL of 10mM NaOH was added to make a 1-10 dilution.

2.2.11.3 High performance liquid chromatography (HPLC) analysis

Carbohydrates were analysed using a DIONEXICS 3000 (Thermo Scientific). Reagent-free ion chromatography equipped with an auto injection system (AS autosampler) and coupled to Dionex ICS-3000 system, electrochemical

detection (ED 40) and computer controller. A CarboPac PA 20 column (3 x 150 mm) was used for the separation with a mobile phase of 10mM NaOH and a flow rate of 0.5 ml/min. The injection volume was 10 µl and the column temperature was 30^{o}C. The carbohydrates were identified by comparing their retention time with standard solutions. Peak areas were calculated using Thermo Scientific Chromeleon software. The concentration of carbohydrate was determined using a standard curve.

2.2.12 Measurement of volatile compounds

2.2.12.1 Sample preparation

Coffee samples were ground to a powder using a mini chopper (CH180 mini chopper, 300watt, Kenwood). 2 g of each sample powder and 2.5 µL of 1% heptanone (internal standard) were placed GC vials for analysis (Yang et al., 2016).

2.2.12.2 Gas chromatography–mass spectrometry (GC-MS) analysis

A GC-ISQ (Thermo Electron Corporation Inc.) was used to detect the volatile compounds of coffee in full scan mode over the mass range 20-200 m/z (ion source 200°C). The sample was incubated at 55°C for 5 min with shaking using the autosampler. A 50/30 µm DVB/Carboxen/PDMS was the fibre sampled the volatiles for 3.5 min followed by a 1.5 min desorption (inlet temperature 200°C). A splitless mode was used and the constant carrier pressure was at 103kPa.

The GC oven was held at 40°C for 5 min, and then increased from 40°C to 240°C at a rate of 5°C/min, and held at 240°C for 2 min. Separation was

carried out on a ZB-WAX Capillary GC Column (Length 30 m, inner diameter 0.25 mm, and film thickness 0.25 μm; Phenomenex Inc., Macclesfield, UK). The identity and quantity of volatile compounds was determined using Thermo Xcalibur software. The mass spectrum was used to identify the volatile compounds by comparing with the library (NIST/EPA/NIH Mass Spectral Library. Version 2.0, Faircom Corporation, U.S.) in Xcalibur software. The peak areas of compounds and the internal standard were determined using Thermo Xcalibur software (Yang et al., 2016). The following formula could be used to calculate the concentration of volatile compounds:

$$The\ concentration\ of\ volatile\ compounds\ (mg/kg) = \frac{Peak\ area\ of\ volatile\ compound \times Amount\ of\ internal\ standard(mg)}{Peak\ area\ of\ internal\ standard \times Dry\ weight\ of\ coffee\ powder(kg)}$$

2.3 Statistical analysis

The experimental design consisted of shade types as main plot and subplots represented by managements but with an unbalanced structure since not all managements were represented under all Shade types (Table 2.1). Therefore, the specific pre-planned contrast models (Modified from Haggar et al., 2011) (Table 2.5) were used to compare the differences between different managements and shade types.

Data was analysed using One-Way ANOVA contrasts model with SPSS statistics software (Version 22nd, IBM), treatment as contrast factor to analyse eight contrast groups as shown in Table 2.5. Significant value ($P<0.05$) in contrast tests was used to decide if there was a significant difference in each contrast model and the standard error of each contrast could be obtained from contrast tests table. In addition, the mean value of every management and shade type under each contrast model was calculated.

The main methodologies have been introduced in this chapter. In the next chapter (Chapter 3), the relationship between the fertilizer levels and shade types of coffee plants and the coffee yield and coffee tree morphology is investigated.

Table 2.5 Principal contrasts used in the analysis of Shade type and Management effects

Contrast	Treatments compared
Management	
IC vs. MC	IC(FS, E, T, CE) vs. MC(FS, E, T, CE)
MC vs. IO	MC(E, T, C, CE, CT, ET) vs. IO(E, T, C, CE, CT, ET)
IO vs. LO	IO(E, CE) vs. LO(E, CE)
IC vs. IO	IC(E, T, CE) vs. IO(E, T, CE)
Shade type	
FS vs. shaded	FS(IC, MC) vs. E(IC, MC) + T(IC, MC) + CE(IC, MC)
Erythrina vs. FS*	E(IC, MC) vs. FS(IC, MC)
Service vs. timber trees	E(MC, IO) vs. T(MC, IO) + C(MC, IO) + TC(MC, IO)
Legume timber vs. non-legume timber	C(MC, IO) vs. T(MC, IO)

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, E: *Erythrina poepiggiana*, C: *Chloroleucon eurycyclum*; T: *Terminalia Amazonia*; CE: *Chloroleucon eurycyclum* and *Erythrina poepiggiana*; CT: *Chloroleucon eurycyclum* and *Terminalia Amazonia*; ET: *Erythrina poepiggiana* and *Terminalia Amazonia*;

* Erythrina was regarded as a low canopy tree with low shade cover and compared with full sun.

Chapter 3 Impact of fertilizer levels and shade types of coffee plants on the coffee yield and coffee tree morphology

3.1 Introduction

A large number of researches have been undertaken into the fertilizers of coffee. Nitrogen, phosphate and potassium have been supposed to be the main nutrition for coffee growth (Willson, 1985). Nitrogen takes responsible for the development of woods, leaves and fruits. The application of nitrogen can increase vegetative growth and yield, the number of nodes per branch, the number of fruiting nodes and the number of flowers per node (Mitchell, 1988). Phosphate is considered as the main mineral for the growth of young coffee plant and it improves the growth rate of seedlings (M'Itungo and van der Vossen, 1981). Potassium plays an essential role in the development of the fruits (Willson, 1985).

In general, due to the slow decomposition and release of organic matter, coffee grown under organic conditions has previously been reported to produce lower yields and worse coffee plant morphology when compared to conventional conditions in Costa Rica, but a subgroup of farms showed a similar or even higher productivity when using organic / agroforestry approaches (Lyngbaek et al., 2001).

Agro-forestry systems as a substitution to full sun production are considered to have a number of advantages including reduction of erosion and nitrogen leaching (DaMatta, 2004; Tully et al. 2012), protection of soil and water resources (Beer et al., 1997), higher carbon storage as well as higher local

biodiversity (Haggar et al., 2011; Tscharntke et al., 2011), buffering of climate extremes (Lin, 2007), enhanced resource capture, such as light (Taugourdeau et al., 2014) and a lower demand of external inputs (DaMatta, 2004). Legume shade tree has been further reported to replace low external nutrient input by N_2 fixation (Nygren et al., 2012). Moreover, DaMatta (2004) and Vaast et al. (2005) stated that under the sub-optimum site condition, coffee plants under the shaded systems had the higher yield than under the full sun system. Nevertheless, the competition for growth resources such as light, water and nutrients between coffee plants and shade trees could be serious disadvantages of agro-forestry systems. In addition, regular pruning and thinning of shade trees also increased the labour cost (Mitchell, 1988).

As a result, in this chapter, the impact of agro-forestry systems including fertilizer levels (organic and non-organic) and shade types on coffee yield, BI yield, pruning intensity and morphology of coffee plant and shade cover are presented.

3.2 Results

3.2.1 Coffee yield and pruning intensity

The integrated mean coffee yield, bienniality index (BI) of coffee yield, integrated mean pruning of coffee plant and shade cover in the different managements and shade types were showed in Table 3.1.

The integrated mean coffee yield was significantly higher ($P<0.05$) under the intensive conventional (IC) management (8.9 t/ha/year for IC vs MC group and 8.1 t/ha/year for IC vs IO group) than under the moderate conventional (MC) (6.2 t/ha/year) and intensive organic (IO) (5.6 t/ha/year) managements. No significant differences could be found between the moderate conventional (MC) and intensive organic (IO) managements and the intensive organic (IO) and low organic (LO) managements. The full sun system (10.4 t/ha/year) had a significantly higher ($P<0.05$) integrated mean coffee yield than the shaded (6.6 t/ha/year) and *Erythrina* (8.0 t/ha/year) systems. In addition, the integrated mean coffee yield was significantly higher ($P<0.05$) under the service shade tree system (6.5 t/ha/year) than under the timber shade tree system (4.8 t/ha/year), while there was not significantly different in the integrated mean coffee yield between the legume timber shade tree and the non-legume timber shade tree systems.

The bienniality index of coffee yield (BI yield) was significantly higher ($P<0.05$) under the intensive conventional (IC) management (7.2 for IC vs MC group and 6.8 for IC vs IO group) than under the moderate conventional (MC) (6.0) and intensive organic (IO) (5.1) managements. Moreover, the BI yield was significantly higher ($P<0.05$) under the moderate conventional (MC)

management (5.7) than under the intensive organic (IO) management (4.6), while no significant difference was detected in the BI yield between the intensive organic (IO) and low organic (LO) managements. The full sun system (8.0) had significantly higher ($P<0.05$) BI yield than the shaded (6.1) and *Erythrina* (6.2) systems, whilst there were no significant differences in the BI yield between the service shade tree and timber shade tree systems and the legume timber shade tree and the non-legume timber shade tree systems.

The integrated mean pruning of coffee plants was significantly higher ($P<0.05$) under the intensive conventional (IC) management (55.0%) than under the moderate conventional (MC) management (48.1%), while no significant differences were found in other contrast groups.

There was not significantly different in the shade cover of coffee plants between the different managements. The shade cover of coffee plants was significantly lower ($P<0.05$) under the service shade tree system (18.4%) than under the timber shade tree system (56.0%), while the legume timber shade tree system (63.1%) had a significantly higher ($P<0.05$) shade cover than the non-legume timber shade tree system (51.7%).

Table 3.1 Contrast results for the variables integrated mean coffee yield (t/ha/year), bienniality index (BI) of coffee yield, integrated mean pruning (%) and shade cover (%). Values are presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (p-value). P-values < 0.05 are printed in bold.

Contrast	Coffee yield (t/ha/year)			BI yield			Pruning (%)			Shade cover (%)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	8.9	0.6	**<.001**	7.2	0.5	**0.024**	55.0	2.4	**0.004**	37.2	2.7	0.242
	6.2			6.0			48.1			40.4		
MC vs IO	5.3	0.3	0.337	5.7	0.4	**0.010**	49.4	1.9	0.459	45.9	1.9	0.907
	5.0			4.6			47.9			46.1		
IO vs LO	5.8	0.6	0.104	4.8	0.7	0.172	49.1	3.2	0.183	32.6	3.3	0.742
	4.8			3.8			44.8			33.7		
IC vs IO	8.1	0.6	**<.001**	6.8	0.6	**0.005**	55.2	2.8	0.060	37.2	2.7	0.573
	5.6			5.1			49.8			38.8		
Shade types												
FS vs S	10.4	0.9	**<.001**	8.0	0.6	**0.002**	53.2	2.8	0.480	-	-	-
	6.6			6.1			51.0			-		
E vs FS	8.0	1.0	**0.020**	6.2	0.7	**0.017**	50.1	3.4	0.399	-	-	-
	10.4			8.0			53.2			-		
Ser. vs Tim.	6.5	0.5	**0.001**	5.3	0.6	0.999	47.3	2.7	0.370	18.4	2.7	**<.001**
	4.8			5.3			49.8			56.0		
LT vs NLT	4.9	0.6	0.889	4.8	0.7	0.149	51.0	3.4	0.995	63.1	3.3	**0.001**
	5.0			5.9			50.8			51.7		

Fig. 3.1 and 3.2, according to the contrast models in Table 2.5, showed the mean coffee yield and mean pruning intensity (%) of coffee plants per year under different managements and shade types.

There were significant effects of year, management techniques and shade types on coffee yield and pruning intensity ($p<0.05$). Conventionally managed

treatments (IC, MC) and organically managed ones (IO, LO) reached their close to maximum productivity in 2003 and 2005, respectively (Fig. 3.1 (a) and (c)). Similarly, conventional managements (IC, MC) showed a higher pruning intensity in the first year of measurements (2004) when compared to IO treatments (Fig. 3.1 (b) and (d)). The yield of LO under the timber species *Terminalia* (TLO) began to fail in 2008 and collapsed totally in 2010 (Fig. 3.1 (c)).

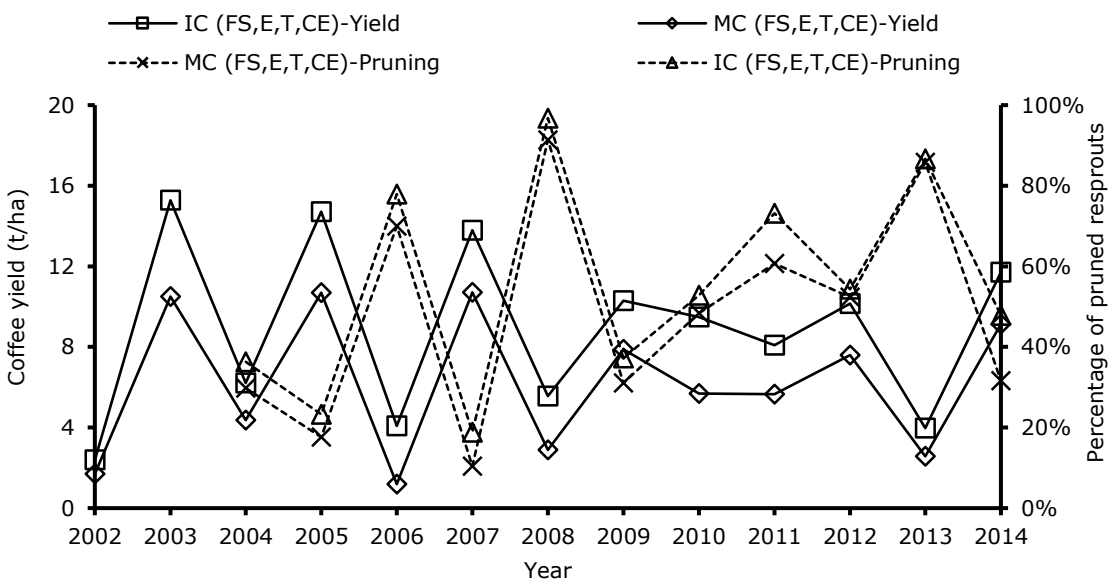
A biennial bearing pattern could be observed for all managements in some years, but fluctuations were stronger for conventional ones. Bienniality index (BI) was significantly higher under the intensive conventional (IC) management than under the moderate conventional (MC) and the intensive organic (IO) managements, with BI being also significantly higher under the moderate conventional (MC) than the intensive organic (IO) managements (Table 3.1). While conventional management showed a clear biennial yield pattern in the first 8 years of production, organic coffee yield rose steadily in the first 4 years until entering a biennial phase (Fig. 3.1 (a) and (c)). From 2009 to 2012 all managements entered a more stable phase with medium yields. These fluctuations led to higher yields in the conventionally managed treatments in years of high yields but in years of low yields similar or even higher yields could be observed in the organically treated ones (Fig. 3.1 (b) and (d)).

Shade and full sun coffee began producing high yields in the same years (2003) i.e. shade type did not affect the onset of production (Fig. 3.2 (a)). Yield fluctuations were larger for full sun coffee when compared to shaded systems

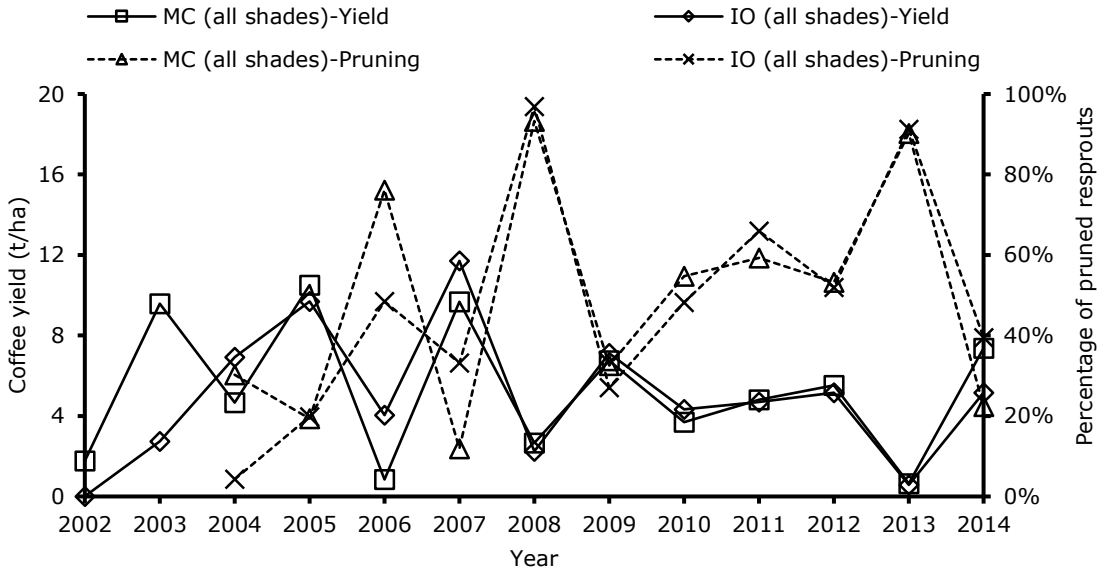
(Fig. 3.2 (a) and (b)). In accordance, BI was significantly higher for full sun than shade, whereas no significant differences could be detected for BI in between the different shade systems (Table 3.1 and Fig. 3.2 (c) and (d)).

Full sun coffee out yielded shade coffee in years of high yields in the biennial phase (2002-2009) and in the stable yield phase (2009-2012), while in years of low yields (biennial phase), performance of shaded coffee was similar (Fig. 3.2 (a) and (b)). Furthermore, shade systems with the high canopy timber trees (*Chloroleucon* and *Terminalia*) showed a similar or even higher yield than systems with the low canopy service tree species *Erythrina* until 2007 but from 2008 onwards systems with *Erythrina* appeared to outperform these high canopy systems (Fig. 3.2 (c)).

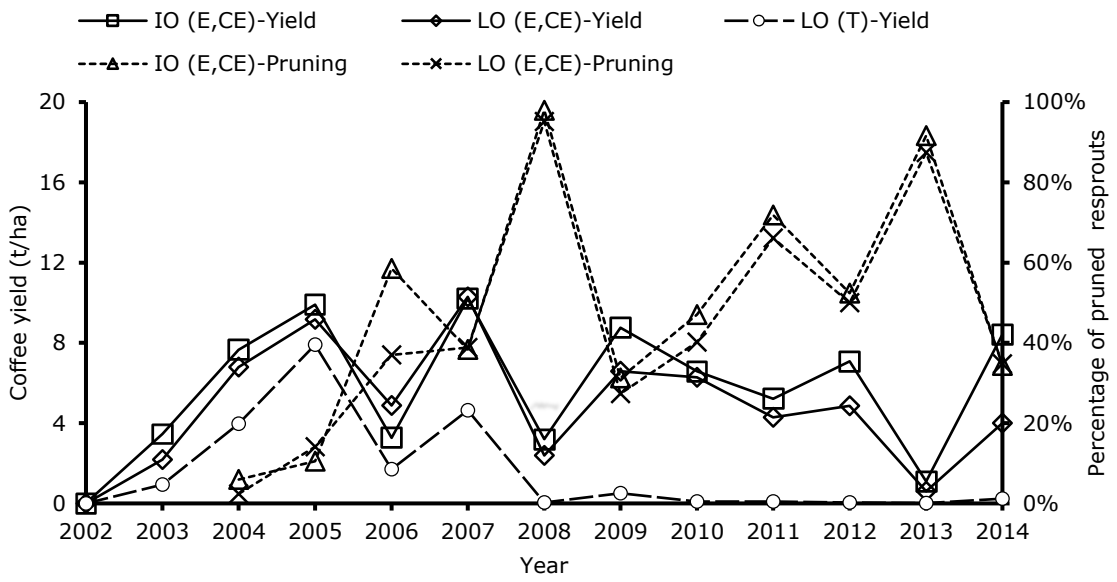
Years of high coffee yields were always followed by high pruning intensities in the subsequent year, especially in the period until 2009 (Fig. 3.1 and 3.2). All treatments experienced three drastic falls in coffee yield (2006, 2008, 2013), especially in 2013 (coffee rust outbreak), that were preceded by very high pruning percentages in the same year (in February-March) (Fig. 3.1 and 3.2). These falls and the following recovery (2007, 2009, and 2014) were observed in all management and shade systems.


IC (FS,E,T,CE)-Yield
MC (FS,E,T,CE)-Yield
MC (FS,E,T,CE)-Pruning
IC (FS,E,T,CE)-Pruning
Coffee yield (t/ha)
Percentage of pruned resprouts
Year
0
4
8
12
16
20
0%
20%
40%
60%
80%
100%
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

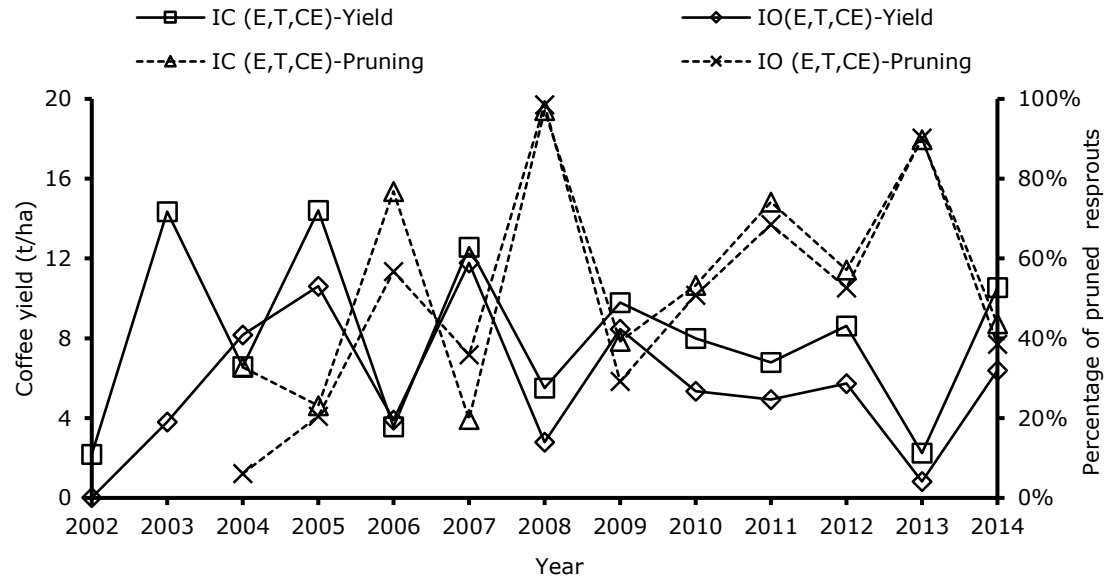
(a)


MC (all shades)-Yield
IO (all shades)-Yield
MC (all shades)-Pruning
IO (all shades)-Pruning
Coffee yield (t/ha)
Percentage of pruned resprouts
Year
0
4
8
12
16
20
0%
20%
40%
60%
80%
100%
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

(b)

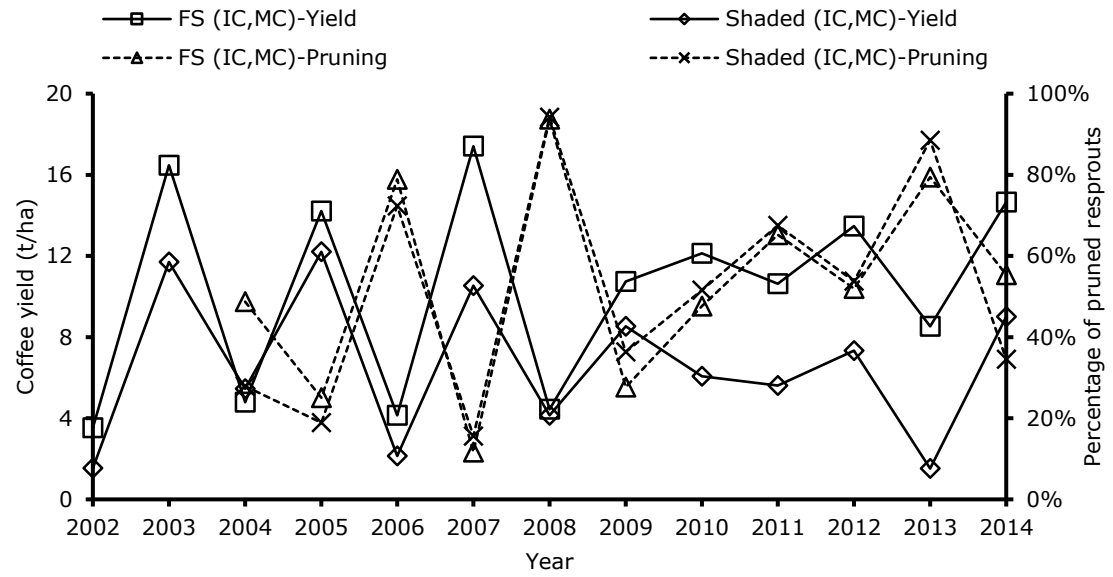


(c)

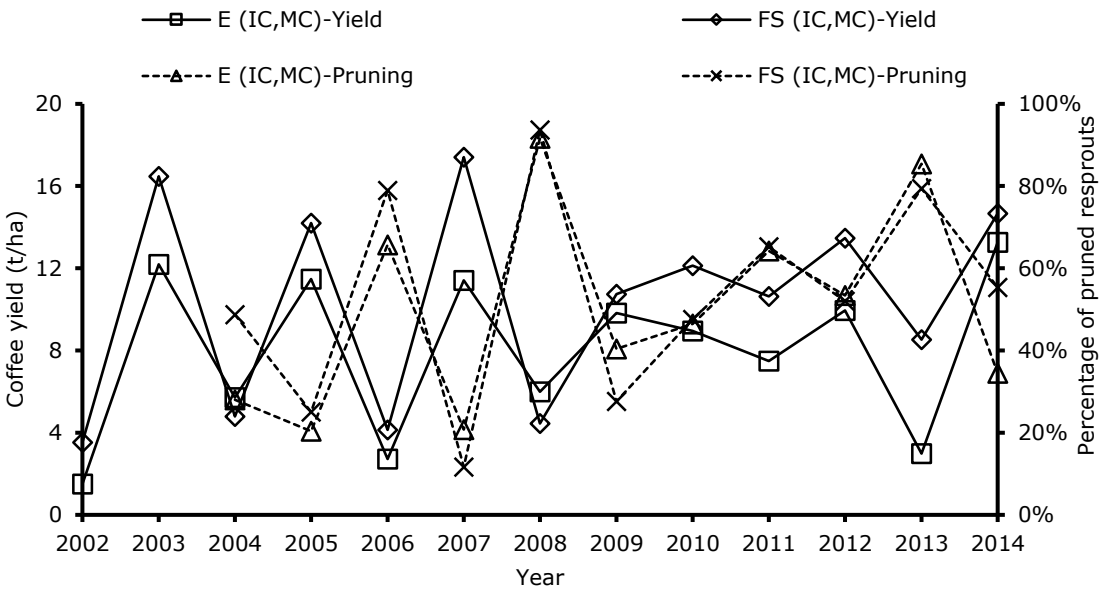


(d)

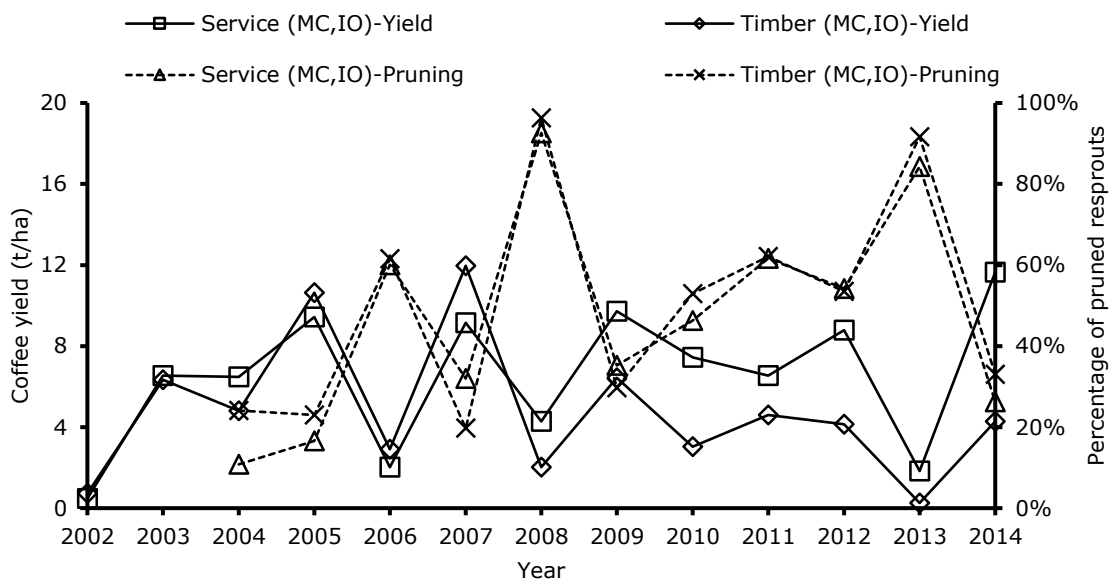
Fig. 3.1 Mean coffee yield and mean pruning intensity (%) of coffee resprouts per year under different managements and same shade types. A detailed contrast description can be found in Table 2.5.


FS (IC,MC)-Yield
Shaded (IC,MC)-Yield
FS (IC,MC)-Pruning
Shaded (IC,MC)-Pruning
Coffee yield (t/ha)
Percentage of pruned resprouts
Year
0
4
8
12
16
20
0%
20%
40%
60%
80%
100%
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

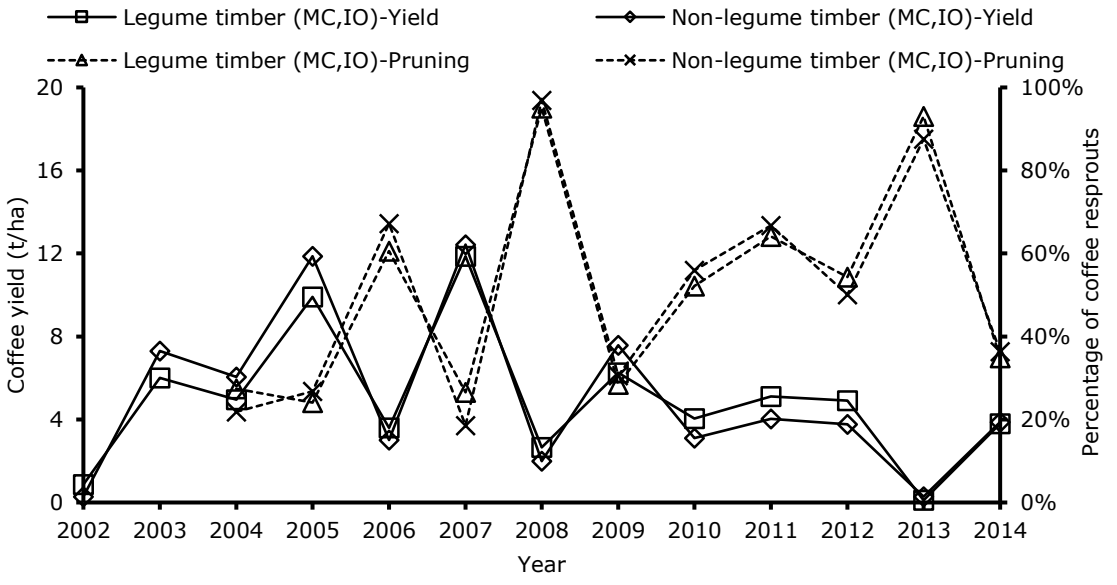
(a)


E (IC,MC)-Yield
FS (IC,MC)-Yield
E (IC,MC)-Pruning
FS (IC,MC)-Pruning
Coffee yield (t/ha)
Percentage of pruned resprouts
Year
0
4
8
12
16
20
0%
20%
40%
60%
80%
100%
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

(b)



(c)



(d)

Fig. 3.2 Mean coffee yield and mean pruning intensity (%) of coffee resprouts per year under different shade types and same managements. A detailed contrast description can be found in Table 2.5.

3.2.2 Coffee morphology

The contrast results for coffee morphology including height (H), diameter (D), total branches (TB) and productive branches (PB) in 2002 and 2014 were shown in Table 3.2, TLO was excluded as a failed system and most plants were dead in 2014.

In 2002, the height of coffee plants was significantly higher ($P<0.05$) under the moderate conventional (MC) management (96 cm) than under the intensive organic (IO) management (75 cm). The coffee plants under the intensive conventional (IC) management (102 cm) had a significantly higher ($P<0.05$) height than those under the intensive organic (IO) management (78 cm). No significant differences were found in the height of coffee plants between the different shade types. However, in 2014, the height of coffee plants was significantly higher ($P<0.05$) under the intensive conventional (IC) management (184 cm for IC vs MC group and 183 cm for IC vs IO group) than under the moderate conventional (MC) (168 cm) and intensive organic (IO) (166 cm) managements. In addition, the intensive organic (IO) management (177 cm) had a significantly higher ($P<0.05$) height of coffee plants than the low organic (LO) management (156 cm). The height of coffee plants was significantly higher ($P<0.05$) under the service shade tree system (184 cm) than under the timber shade tree system (154 cm).

In terms of the effect of different managements on the root diameter of coffee plants, in 2002, the root diameter of coffee plants was significantly bigger ($P<0.05$) under the intensive conventional (IC) management (23 cm for both IC vs MC and IC vs IO groups) than under the moderate conventional (MC) (21

cm) and intensive organic (IO) (15 cm) managements. The moderate conventional (MC) (20 cm) management had a significantly bigger ($P<0.05$) root diameter of coffee plants than the intensive organic (IO) (14 cm) management. In 2014, similar results were found in the IC vs MC and IC vs IO groups, while there was not significantly different in the root diameter of coffee plants between the moderate conventional (MC) and intensive organic (IO) managements. Moreover, the root diameter of coffee plants was significantly bigger ($P<0.05$) under the intensive organic (IO) management (21 cm) than under the low organic (LO) management (18 cm). For the effect of different shade types on the root diameter of coffee plants, in 2002, the root diameter of coffee plants was significantly bigger ($P<0.05$) under the full sun system (24 cm) than under the shaded system (21 cm), while in 2014, the service shade tree system (23 cm) had a significantly bigger ($P<0.05$) root diameter than the timber shade tree system (19 cm).

With regards to the total branches, in 2002, the total branches of coffee plants was significantly more ($P<0.05$) under the intensive conventional (IC) management (40 for IC vs MC group and 37 for IC vs IO group) than under the moderate conventional (MC) (33) and intensive organic (IO) (15) managements. The moderate conventional (MC) management (30) had a significantly more ($P<0.05$) total branches of coffee plants than the intensive organic (IO) management (13). In 2014, similar results were found in the IC vs MC and IC vs IO groups, while there was not significantly different in the total branches of coffee plants between the moderate conventional (MC) and intensive organic (IO) managements. Moreover, the total branches of coffee plants were significantly more ($P<0.05$) under the intensive organic (IO)

management (39) than under the low organic (LO) management (33). For the effect of different shade types on the total branches of coffee plants, in 2002, the total branches of coffee plants were significantly more ($P<0.05$) under the full sun system (47) than under the shaded system (33), while in 2014, the service shade tree system (43) had a significantly more ($P<0.05$) total branches than the timber shade tree system (34).

The productive branches of coffee plants only were measured in 2014. The productive branches of coffee plants was significantly more ($P<0.05$) under the intensive conventional (IC) management (42 for both IC vs MC and IC vs IO groups) than under the moderate conventional (MC) (37) and intensive organic (IO) (34) managements. The productive branches of coffee plants under the intensive organic (IO) management (33) were significantly less ($P<0.05$) than under the moderate conventional (MC) management (36), while they were significantly more ($P<0.05$) under the intensive organic (IO) management (37) than under the low organic (LO) management (28). For shade types, the *Erythrina* system (44) had a significantly more ($P<0.05$) productive branches than the full sun system (41), whilst the service shade tree system (41) had a significantly more ($P<0.05$) productive branches than the timber shade tree system (31).

Table 3.2 Contrast results for coffee morphology: The variables height (H), root diameter (D), total branches (TB) and productive branches (PB) for 2002 and 2014. Values are presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (p-value). P-values < 0.05 are printed in bold. The used contrasts were explained in detail in Table 2.5.

Contrast	Height 2002 (cm)			Height 2014 (cm)			Diameter 2002 (cm)			Diameter 2014 (cm)			Total Branches 2002			Total Branches 2014			Prod. Branches 2014		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	104	4.2	0.132	184	4.2	**<.001**	23	1.3	**0.030**	22	0.8	**0.016**	40	4.1	**0.070**	45	1.4	**<.001**	42	1.1	**<.001**
	97			168			21			21			33			39			37		
MC vs IO	96	3.4	**<.001**	164	3.4	0.714	20	1.0	**<.001**	20	0.6	0.464	30	2.6	**<.001**	38	1.2	0.055	36	1.3	**0.031**
	75			163			14			20			13			36			33		
IO vs LO	80	5.9	0.309	177	5.9	**0.001**	15	1.8	0.397	21	1.1	**0.003**	16	3.9	0.653	39	2.1	**0.005**	37	2.6	**0.002**
	74			156			14			18			14			33			28		
IC vs IO	102	4.8	**<.001**	183	4.8	**<.001**	23	1.5	**<.001**	23	0.9	**0.008**	37	4.0	**<.001**	45	1.6	**<.001**	42	1.6	**<.001**
	78			166			15			20			15			37			34		
Shade types																					
FS vs S	107	4.8	0.092	180	4.8	0.263	24	1.5	**0.029**	21	0.9	0.503	47	5.4	**0.010**	43	1.6	0.529	41	1.2	0.143
	98			175			21			22			33			42			39		
E vs FS	103	5.9	0.558	183	5.9	0.651	21	1.8	0.067	23	1.1	0.159	36	6.3	0.075	46	1.9	0.107	44	1.3	**0.049**
	107			180			24			21			47			43			41		
Ser. vs Tim.	92	4.8	0.114	184	4.8	**<.001**	19	1.5	0.087	23	0.9	**<.001**	27	3.9	0.072	43	1.6	**<.001**	41	1.4	**<.001**
	84			154			16			19			19			34			31		
LT vs NLT	85	5.9	0.695	156	5.9	0.204	17	1.8	0.756	19	1.1	0.873	20	4.3	0.731	34	2.1	0.898	31	2.7	0.580
	83			148			16			19			19			34			30		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber; Prod. branches: productive branches.

3.2.3 Shade cover

Shade cover (%) in 2014 differed significantly ($p<0.05$) between the different agro-forestry systems (Fig. 3.3). When the different managements were contrasted for the same shade types (Table 2.5) no significant difference was found, while significant differences in shade cover existed between the shade tree species combinations (Table 3.1). The service tree species *Erythrina* had a lower shade cover than the timber tree species (*Chloroleucon* and *Terminalia*) and their combinations (18.4 vs 56.0 %) (Table 3.1).

Moreover, shade cover was significantly higher under *Chloroleucon* than under *Terminalia* (51.7 vs. 63.1 %) (Table 3.1). In general, shade cover in mixed species systems that incorporated *Erythrina* was always lower than in the corresponding timber tree monocultures (Fig. 3.3).

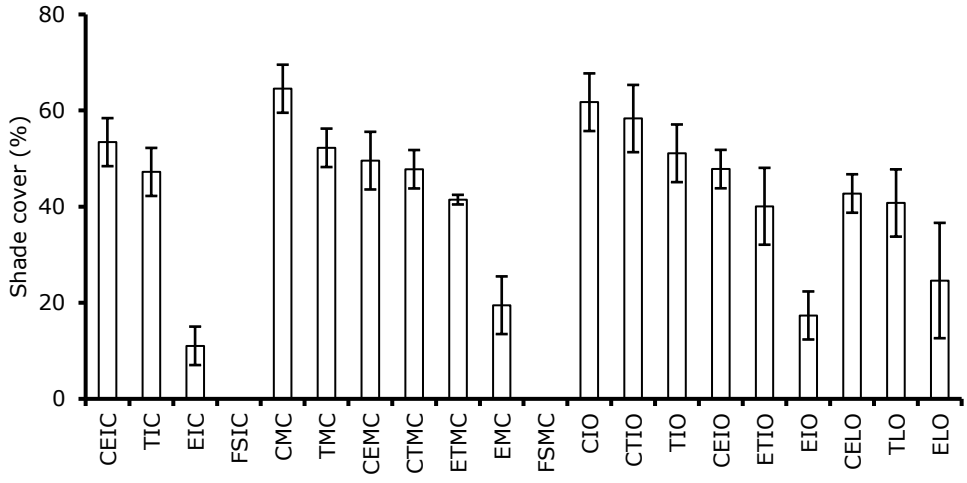


Fig. 3.3 Shade cover (% mean ± SD) under the different agro-forestry systems in 2014.

3.3 Discussions

3.3.1 Effects of management on yield. In what circumstances can organic compete with conventional?

It is generally stated that the coffee yield in the organic management is lower than in the conventional management in coffee agriculture (Blackman and Naranjo, 2012). In our experiment, although the intensive organic (IO) management received higher phosphorus (P) and potassium (K) inputs and only slightly lower total nitrogen (N) input (see Table 2.4 in the chapter 2), the intensive organic (IO) productivity including yield and morphology was always lower than the intensive conventional (IC) management, however, it was similar to the moderate conventional (MC) management which received half the amount of intensive conventional (IC) management fertilizer. Thus, the organic coffee with high productivity could be achieved, although its productivity still remained below the intensive conventional (IC) management.

Compared with the moderate conventional (MC) and organic managements, the intensive conventional (IC) management had a better performance due to the slower release of plant available N from organic inputs (Seufert et al., 2012), better availability of chemical fertilizers and the positive correlation of coffee yield and N-fertilizer input (Castro-Tanzi et al., 2012).

In our experiment, in the first 2 years of production (2002 and 2003), the coffee productivity including yield and morphology under the moderate conventional (MC) management was better than under the intensive organic (IO) management, due to the longer release period of organic fertilizers and the time needed for soil organic matter recovery after the previous management of the

plots as sugar cane plantation (Haggar et al., 2011). However, after the third year of production (2004), the intensive organic (IO) management had a similar production to the moderate conventional (MC) management, due to the increasing productivity over time in organic agriculture (Seufert et al., 2012). As a result, the mean 13-years coffee yield were similar between the moderate conventional (MC) and intensive organic (IO) managements. To conclude, in terms of the productivity, the intensive organic (IO) management may be used to replace the moderate conventional (MC) management. In addition, according to the international coffee organization (ICO) reports, the coffee price fluctuates annually due to the recurring abiotic calamities such as frosts and droughts. Thus, the cost of intensive conventional (IC) management will be the main limiting factor to coffee farmers, when coffee price drops. The intensive organic (IO) management, therefore, may be an alternative choice to cope with this situation.

3.3.2 Comparing full sun and shaded treatments for yield and morphological variables

Compared with the full sun system, the shaded systems had the significantly lower ($P<0.05$) mean coffee yield in the 13 years, while in contrary to coffee yield, the morphology, including height, root diameter, total branches and productive branches of coffee plants, were similar between these systems in 2014. The full sun system has the higher yield than the shaded systems due to the higher light availability and the nutrients competition between the coffee plants and shade trees (Vaast et al., 2005; DaMatta, 2004). The lower light intensity in the shaded systems restricted the nodal and flower bud

development (Beer et al., 1997; Campanha et al., 2004) and consequently coffee yield, while the vegetative development of coffee plants such as height, diameter and number of branches was improved, which led to the similar vegetative development under the shaded systems (Morais et al., 2003; Vaast et al. 2005). However, in the full sun system, the increase of productivity is achieved by using a large number of external agrochemical inputs and shortcutting ecological cycles (Beer et al., 1997; Haggar et al., 2011) which contributes to environmental pollution, degradation of soils (Lyngbaek et al., 2001; DaMatta, 2004) and health hazards (Nygern, 2012). The high annual cost caused by these high external inputs cannot be reduced easily, which results in vulnerability of coffee farmers to the unstable international coffee market (Haggar et al., 2011). Shaded system, therefore, is considered as the essential option for a sustainable coffee production, as it improves adverse site conditions such as temperature, humidity and soil conditions (de Carvalho Gomes et al., 2016; Lin, 2007) and even resulting in similar or even higher yields under shaded systems (DaMatta, 2004; Vaast et al., 2005). Furthermore, shaded system also maintains the production stability due to the smaller fluctuation and lower bienniality index (BI) of coffee yield.

3.3.3 The changing performance of service and timber tree species

The mean coffee yield in the 13 years was significantly higher ($P<0.05$) under the service tree species *Erythrina* than under the timber tree species *Chloroleucon* and *Terminalia*. However, annual coffee yield was quite different in the initial and late development stage of the coffee plants between these two shade tree systems (see Fig. 3.2 (c)). In the first 6 years of production, the

coffee plants under the timber shade tree systems had the similar or even higher annual coffee yield than those under the service shade tree system, while from the 7th year onwards, this pattern shifted completely to clearly higher yields and improved coffee morphology under the service shade tree system. As a result, Haggar et al. (2011) who analysed the data in the first 6 years of production, drew the conclusion that the timber trees might be the more favorable option in coffee agro-forestry systems, due to the higher yield and increasing revenue of timber sales. In addition, the service shade tree (*Erythrina*) had the lower coffee yield due to the higher shade cover prior to pruning and higher competition between the shade trees and coffee plants (*Erythrina* had the larger basal area).

However, in the long term, the performance of service shade tree systems was better than timber shade tree systems. There were some reasons to explain this phenomenon. First of all, the service tree (*Erythrina*) was pruned heavily every year, whilst only the lower branches of timber trees were pruned, which led to the sustainable decreasing shade cover for the service tree and the similar or even higher shade cover for the timber trees over time. Secondly, the expanding growth of the timber tree crowns increased the competition between the timber trees and coffee plants. Finally, compared with the timber tree system, service tree system had the higher biomass inputs and nutrient recycling through severe pruning and litter fall (Boreux et al., 2016).

3.3.4 Compensation effects of legume trees

There was no significant difference detected in the coffee yield between the intensive organic (IO) and low organic (LO) managements when combined

with the legume species *Erythrina* and *Chloroleucon*, while the low organic (LO) management under the non-legume shade tree species (*Terminalia*) collapsed totally (see Fig. 3.1 (c)). On the one hand, legume tree species can compensate the low external input for the low organic (LO) management by N_2 fixation (about 70-90 kg N /ha/year) (Tully and Lawrence, 2011; Nygren et al., 2012); on the other hand, the legume species especially *Erythrina* can replenish the organic matter to the soil through extremely strong pruning (the leaves and branches of shad trees were left on the field after pruning) (Haggar et al., 2011). The very low N-availability of coffee plants under the non-legume tree species *Terminalia* might be a reason to explain the collapse of coffee plants in this system. As a result, the legume shade tree had positive effects on coffee productivity especially in the low external inputs management. Moreover, the production of coffee plants under the non-legume shade tree system largely depended on the input amounts of the external fertilizer.

3.3.5 Implications for coffee producers and ecosystem services

Besides the overall yields, for coffee farmers, the production stability, which is related to the stable income of coffee farmers, is another crucial factor when they choose the planting system. Although providing the highest overall yields, the full sun system and the intensive conventional (IC) management had the highest yield bienniality especially in the first 8 years. Biennial production was positively correlated with pruning intensity which would impact the overall labour cost. It has been commonly reported that the full sun system had the stronger biennial production than the shaded system (DaMatta, 2004; Vaast et al., 2006).

The most probable explanation for the yield bienniality is plant exhaustion. The coffee plants grown under the full sun or the intensive conventional (IC) condition produce high cherry loads at the cost of vegetative development which exhausts the reserves of the coffee plants and leads to the low yields in the subsequent year (the coffee plant will recover the growth of branches and leaves in the following year) (DaMatta, 2004). The higher exhaustion and fluctuation decreases the life span of coffee plants (DaMatta, 2004), which, in some sense, increases the coffee farmer's cost. In addition, the high cost of external inputs in the intensive conventional (IC) management is also a limiting factor for coffee farmers to choose the coffee planting system especially when coffee prices fall. It is suggested that economically viable production can be maintained when applying moderate doses of fertilizers (Castro-Tanzi et al., 2012). For the shaded system with the timber trees, timber sales can also increase the income for coffee farmers. Therefore, for coffee producers, the shaded systems with the moderate conventional or organic managements may be an alternative option for achieving the lower cost.

The coffee production has a significant effect on the environment, which is the essential concerns for the government. In our experiment, the level of N fertilizer was found to be the main reason for greenhouse gas emissions, with less emissions at lower inputs and organic managements (Noponen et al., 2012). Additionally, greenhouse gas emissions in all agroforestry systems could be fully compensated by the carbon storage in above and below ground tree biomass (Noponen et al., 2013).

Finally, compared with the conventional management, organic management

may decrease the cost due to lower price of organic fertilizers such as chicken manure and coffee pulp (Blackman and Naranjo, 2012) and lead to better soil characteristics like higher soil organic matter content (Haggar et al., 2011). Nevertheless, lower yield (Seufert et al., 2012) with a relatively low price premium of 10 - 20% and associated certificate cost (Blackman and Naranjo, 2012) of organic management restrict its application. In our experiment, compared with the moderate conventional (MC) management, both organic managements had the similar or even higher production when legume shade tree was used. As a result, when the high cost of the intensive conventional (IC) management is not affordable, organic coffee production may be an alteration.

3.4 Conclusions

The coffee productivity including the yield and morphology was worse under the intensive organic (IO) management than under the intensive conventional (IC) management. However, after a long period, the intensive organic (IO) had a similar or even higher productivity than the moderate conventional (MC) management. Organic management showed a more stable production, which would promote stability of the coffee farmers' income. The coffee yield was significantly lower under the shaded system than under the full sun system, while the performance of coffee morphology was similar between these two systems in 2014. The service shade tree system had a significantly higher coffee yield and better morphology performance than the timber shade tree system. Legume tree species could compensate the insufficient of nutrients inputs for the low organic (LO) management by fixing N_2.

This chapter (Chapter 3) investigated and discussed the effect of different fertilizer levels and shade types of coffee plants on coffee yield and morphology of coffee plants, and the next chapter (Chapter 4) explores the relationship between the different fertilizer levels and shade types of coffee plants and the physical and chemical attributes of green coffee beans.

Chapter 4 Impact of fertilizer levels and shade types of coffee plants on the physical and chemical attributes of green coffee beans

4.1 Introduction

A large number of nutriments such as carbohydrates, proteins, lipids, minerals, organic acids have been reported in the green coffee beans, however, the amounts and varieties of these nutrients strongly depend on the origin of coffee tree and field treatments (Valkila, 2009). In addition, the original and growth conditions of coffee trees in the field also influence the physical properties of green coffee beans such as density, bulk density and moisture content (Dutra et al., 2001).

As mentioned in the introduction (Chapter 1), many physical attributes (green bean density, bulk density and moisture content) and chemical characteristics (minerals, lipids, carbohydrates, protein and caffeine) of green coffee beans have been introduced and the contents of these substances would vary due to the origin of coffee plant species and growth conditions including nutrient input amount, light intensity, temperature and water resource in the field, nevertheless, a few publications connected the physical and chemical attributes of green coffee bean with the agricultural treatments were reported in the last few decades. As a result, it is necessary for coffee scientists to explore the relationships between the species and the growth conditions and the physical and chemical attributes of green coffee bean. In this chapter, the impact of agro-forestry systems including fertilizer levels (organic and non-organic) and

shade types on the physical and chemical attributes of green coffee beans are presented.

4.2 Results

4.2.1 Impact of fertilizer levels and shade types on the physical attributes of green coffee bean

According to the contrast model designed in chapter 2, contrast results for density, bulk density, moisture content and roasting loss were determined (Table 4.1). In terms of density, bulk density, moisture content and roasting loss, there were no significant differences ($P<0.05$) detected between managements. However, when compared with the shaded system, the density of green coffee bean under the full sun system was significantly higher (1462 kg/m^3 in the full sun system and 1372 kg/m^3 in the shaded system, $P<0.05$), while when compared with the full sun system, the density of green coffee bean under the *Erythrina* was significantly lower (1363 kg/m^3 in the *Erythrina* system and 1462 kg/m^3 in the full sun system, $P<0.05$). For bulk density, no significant differences ($P<0.05$) were detected between different shade types.

With regards to the moisture content, the green coffee beans under the full sun system (108 g/Kg) had a significant lower value ($P<0.05$) than under the shaded system (111 g/Kg) and the *Erythrina* system (114 g/Kg). In addition, the moisture content of green coffee beans under the service system (110 g/Kg) was significantly lower ($P<0.05$) than under the timber system (113 g/Kg).

The roasting loss of green coffee beans under the full sun system (125 g/Kg) was significantly lower ($P<0.05$) than in the shaded system (132 g/Kg) and the *Erythrina* (135 g/Kg) system, while the green coffee beans under the timber system (135 g/Kg) presented a significantly higher ($P<0.05$) roasting loss value than under the service system (127 g/Kg).

Table 4.1 Contrast results for density (Kg/ m^3), bulk density (Kg/ m^3), moisture content (g/Kg) and roasting loss (g/Kg); Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Density (kg/m^3)			Bulk Density (kg/m^3)			Moisture content (g/Kg)			Roasting loss (g/kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	1372	78	0.184	671	19	0.531	110	9	0.691	130	9	0.874
	1399			668			111			130		
MC vs IO	1363	95	0.399	669	24	0.362	112	11	0.281	131	11	0.207
	1376			673			110			128		
IO vs LO	1399	55	0.312	670	14	0.891	109	7	0.462	131	6	0.236
	1371			671			112			127		
IC vs IO	1372	67	0.587	670	17	0.941	111	8	0.337	132	8	0.057
	1385			670			108			127		
Shade types												
FS vs S	1462	35	**0.018**	672	34	0.495	108	5	**0.033**	125	6	**0.017**
	1372			668			111			132		
E vs FS	1363	25	**0.024**	667	14	0.503	114	2	**0.002**	135	3	**0.003**
	1462			672			108			125		
Ser. vs Tim.	1381	35	0.335	665	34	0.159	110	5	**0.028**	127	6	**0.002**
	1359			674			113			135		
LT vs NLT	1371	25	0.806	681	14	0.054	111	2	0.276	126	3	0.254
	1364			668			107			122		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

4.2.2Impact of fertilizer levels and shade types on the mineral contents of green coffee bean

Twenty-eight minerals, including macroelements K, Mg, P, S, Ca and microelements Mn, Fe, Cu, Zn, Na, Mo, Co, Ba, Sr, Rb, *etc*., were found in the green coffee beans, while only some main minerals (K, Mg, P, S, Ca, Mn, Fe, Cu, Zn, Na, Mo) were analysed and presented in the Table 4.2 and 4.3. No significant differences (P<0.05) were detected in magnesium (Mg) and iron (Fe)

contents under the different managements and shade types.

With regards to the effect of different managements on the mineral contents of green coffee beans, manganese (Mn) and zinc (Zn) contents were significantly higher ($P<0.05$) under the intensive conventional (IC) (70 mg/Kg, 7 mg/Kg) than under the moderate conventional (MC) (47 mg/Kg, 6 mg/Kg). The contents of manganese (Mn) and sodium (Na) were significantly higher ($P<0.05$) under the moderate conventional (MC) (47 mg/Kg, 5.4 mg/Kg) than under the intensive organic (IO) (22 mg/Kg, 4.9 mg/Kg), whilst the contents of potassium (K), phosphorus (P), calcium (Ca) and molybdenum (Mo) were significantly lower ($P<0.05$) under the moderate conventional (MC) (19 g/Kg, 1.8 g/Kg, 1.4 g/Kg, 54 µg/Kg) than under the intensive organic (IO) (20 g/Kg, 1.9 g/Kg, 1.6 g/Kg, 220 µg/Kg). In addition, the content of calcium (Ca) and molybdenum (Mo) was significantly higher ($P<0.05$) under the intensive organic (IO) (1.6 mg/g, 200 µg/Kg) than the low organic (LO) (1.5 g/Kg, 142 µg/Kg), while sodium (Na) content was significantly lower ($P<0.05$) under the intensive organic (IO) (4.8 mg/Kg) than under the low organic (LO) (5.4 mg/Kg). Furthermore, the green coffee beans with intensive conventional (IC)input revealed significantly more ($P<0.05$) manganese (Mn), copper (Cu) and zinc (Zn) content (62 mg/Kg, 17 mg/Kg, 7 mg/Kg) than those with intensive organic (IO) input (22 mg/Kg, 15 mg/Kg, 6 mg/Kg), while the green coffee beans contained significantly less ($P<0.05$) phosphorus(P) and molybdenum (Mo) content under the intensive conventional (IC) (1.8 g/Kg, 54 µg/Kg) than under the intensive organic (IO) (1.9 g/Kg, 215 µg/Kg).

In terms of the impact of different shade types on the mineral contents of green coffee beans, the contents of calcium (Ca) and manganese (Mn) were significantly higher ($P<0.05$) under the full sun system (1.5 g/Kg, 69 mg/Kg) than under the shaded system (1.4 g/Kg, 55 mg/Kg), whilst the copper (Cu) content was significantly lower ($P<0.05$) under the full sun system (15 mg/Kg) than under the shaded system (16 mg/Kg). Moreover, The copper (Cu), zinc (Zn) and sodium (Na) contents were significantly higher ($P<0.05$) under the *Erythrina* system (18 mg/Kg, 8 mg/Kg, 5.2 mg/Kg) than under the full sun system (15 mg/Kg, 7 mg/Kg, 4.5 mg/Kg), while the contents of sulfue (S), calcium (Ca) and manganese (Mn) were significantly lower ($P<0.05$) under the *Erythrina* system (1.7 g/Kg, 1.3 g/Kg, 50 mg/Kg) than under the full sun system (1.9 g/Kg, 1.5 g/Kg, 69 mg/Kg). No significant differences ($P<0.05$) were found in all mineral contents between the service and timber shade trees, and the legume timber and non-legume timber shade trees.

Table 4.2 Contrast results for majormineral contents (mg/g) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast		K (g/Kg)			Mg (g/Kg)			P (g/Kg)			S (g/Kg)			Ca (g/Kg)	
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	20	1.4	0.292	2.0	0.2	0.651	1.8	0.09	0.251	1.9	0.13	0.088	1.4	0.10	0.059
	20			2.0			1.8			1.8			1.4		
MC vs IO	19	0.7	**<.001**	2.1	0.2	0.813	1.8	0.08	**<.001**	1.8	0.16	0.578	1.4	0.13	**<.001**
	20			2.1			1.9			1.8			1.6		
IO vs LO	20	1.0	0.676	2.1	0.1	0.699	1.9	0.06	0.98	1.8	0.09	0.26	1.6	0.07	**0.003**
	20			2.1			1.9			1.8			1.5		
IC vs IO	20	1.2	0.579	2.0	0.1	0.145	1.8	0.08	**<.001**	1.8	0.11	0.151	1.4	0.09	**<.001**
	20			2.1			1.9			1.8			1.6		
Shade types															
FS vs S	20	2.5	0.323	2.1	0.3	0.205	1.8	0.15	0.654	1.9	0.23	0.283	1.5	0.08	**0.002**
	20			2.0			1.8			1.8			1.4		
E vs FS	19	1.0	0.086	2.0	0.1	0.266	1.7	0.06	0.758	1.7	0.09	**0.003**	1.3	0.07	**<.001**
	20			2.1			1.8			1.9			1.5		
Ser. vs Tim.	19	2.5	0.476	2.1	0.3	0.844	1.8	0.15	0.692	1.7	0.23	0.08	1.5	0.08	0.768
	20			2.1			1.8			1.8			1.5		
LT vs NLT	20	1.0	0.658	2.1	0.1	0.551	1.8	0.06	0.247	1.8	0.09	0.054	1.4	0.07	0.052
	20			2.0			1.8			1.7			1.5		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

Table 4.3 Contrast results for trace mineral contents (mg/Kg and µg/kg) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Mn (mg/Kg)			Fe (mg/Kg)			Cu (mg/Kg)			Zn (mg/Kg)			Na (mg/Kg)			Mo (µg/kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	70	3.7	**<.001**	33	5.2	0.957	17	2.3	0.052	7	0.7	**<.001**	5.1	0.7	0.711	55	18	0.578
	47			32			15			6			5.2			57		
MC vs IO	47	4.5	**<.001**	33	6.3	0.749	16	2.8	0.599	6	1.4	0.082	5.4	0.8	**<.001**	54	22	**<.001**
	22			34			16			6			4.9			220		
IO vs LO	22	2.6	0.134	30	3.7	0.231	15	1.6	0.342	6	0.8	0.671	4.8	0.5	**0.024**	200	13	**<.001**
	24			32			16			6			5.4			142		
IC vs IO	62	3.2	**<.001**	32	4.5	0.197	17	1.0	**0.008**	7	0.8	**<.001**	4.9	0.6	0.874	54	16	**<.001**
	22			30			15			6			4.9			215		
Shade types																		
FS vs S	69	6.3	**<.001**	33	9.0	0.587	15	0.9	**0.006**	7	1.9	0.549	4.5	1.2	0.397	58	31	0.651
	55			32			16			7			4.4			55		
E vs FS	50	2.6	**<.001**	31	3.7	0.299	18	1.6	**<.001**	8	0.8	**0.023**	5.2	0.5	**0.002**	53	13	0.418
	69			33			15			7			4.5			58		
Ser. vs Tim.	35	6.3	0.406	32	9.0	0.381	17	0.9	0.205	6	1.9	0.162	4.8	1.2	0.096	142	31	0.07
	34			34			16			6			5.2			147		
LT vs NLT	34	2.6	0.283	35	3.7	0.223	15	1.6	0.772	6	0.8	0.951	5.0	0.5	0.728	160	13	0.105
	35			32			15			6			5.1			155		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

4.2.3 Impact of fertilizer levels and shade types on the total lipid and fatty acid contents of green coffee bean

The total lipid and fatty acids content were measured and ten fatty acids including palmitic acid (C16:0), Linoleic acid (C18:2), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), oleic acid (C18:1), lignoceric acid (C24:0), linolenic acid (C18:3), cosenoic acid (20:1) and myristic acid (C14:0) were found in the green coffee beans from CATIE (Tropical Agricultural Research and Higher Education Centre), Costa Rica site (Table 4.4 and 4.5).

The total lipid content of green coffee beans was significantly higher ($P<0.05$) under the intensive conventional (IC) (163 g/Kg) than under the moderate conventional (MC) (151 g/Kg). Moreover, the green coffee beans harvested from the intensive organic (IO) field contained significantly more ($P<0.05$) total lipid (162 g/Kg), palmitic acid (37 g/Kg), oleic acid (1.0 g/Kg) and cosenoic acid (44 mg/Kg) than those obtained from the moderate conventional (MC) management, which were 149 g/Kg, 35 g/Kg, 0.8 g/Kg and 38 mg/Kg for the total lipid, palmitic acid, oleic acid and cosenoic acid, respectively. Nevertheless, there were no significant differences ($P<0.05$) detected in the total lipid and all fatty acids contents between the intensive organic (IO) and low organic (LO), and the intensive conventional (IC) and intensive organic (IO).

With regards to the effect of shade types on the total lipid and fatty acids contents, no significantly differences ($P<0.05$) were found between the full sun and shaded system, and the legume timber and non-legume timber system.

However, the total lipid and all fatty acids content were significantly higher ($P<0.05$) under the *Erythrina* system than under the full sun system. Similarly, the total lipid and almost all fatty acids except for the myristic acid content were significantly higher ($P<0.05$) under the service system than under the timber system.

Table 4.4 Contrast results for total lipid and fatty acids contents (g/Kg) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Total lipid (g/Kg)			Palmitic acid (g/Kg)			Linoleic acid (g/Kg)			Stearic acid (g/Kg)			Arachidic acid (g/Kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	163	8	**0.021**	37	0.5	0.368	14	0.2	0.635	10.3	2.7	0.592	8.9	1.8	0.729
	151			36			14			9.9			8.8		
MC vs IO	149	6	**0.003**	35	0.6	**0.033**	13	0.9	0.134	8.9	3.3	0.153	8.4	2.2	0.522
	162			37			14			9.7			8.6		
IO vs LO	147	9	0.428	36	0.3	0.229	14	1.5	0.516	9.8	1.9	0.717	9.1	1.3	0.771
	142			38			14			10.1			8.9		
IC vs IO	152	12	0.3	36	0.4	0.35	14	1.8	0.318	10.2	2.4	0.231	9.0	1.6	0.512
	146			35			13			9.3			8.7		
Shade types															
FS vs S	151	18	0.195	35	0.8	0.114	13	0.4	0.1	9.4	1.7	0.262	8.3	1.2	0.146
	159			37			14			10.3			9.1		
E vs FS	173	14	**<.001**	41	1.2	**<.001**	16	0.2	**0.001**	13.0	1.9	**<.001**	11.0	1.3	**<.001**
	151			35			13			9.4			8.3		
Ser. vs Tim.	167	18	**0.001**	39	0.8	**0.002**	15	0.4	**0.001**	11.9	1.7	**<.001**	10.4	1.2	**<.001**
	146			35			13			8.8			8.2		
LT vs NLT	155	14	0.59	37	1.2	0.267	14	0.2	0.39	9.0	1.9	0.789	8.4	1.3	0.869
	151			35			13			9.3			8.3		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

Table 4.5 Contrast results for fatty acids contents (g/Kg and mg/Kg) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Behenic acid (g/Kg)			Oleic acid (g/Kg)			Lignoceric acid (g/Kg)			Linolenic acid (mg/Kg)			Cosenoic acid (mg/Kg)			Myristic acid (mg/kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	1.9	0.5	0.849	0.9	0.2	0.449	0.71	0.2	0.714	138	33	0.308	43	9	0.247	28	10	0.507
	1.9			0.9			0.70			130			40			28		
MC vs IO	1.8	0.6	0.734	0.8	0.3	**<.001**	0.67	0.2	0.797	133	40	0.868	38	11	**0.003**	28	12	0.836
	1.8			1.0			0.68			134			44			28		
IO vs LO	2.0	0.3	0.245	0.9	0.2	0.475	0.77	0.1	0.3	140	23	0.689	41	7	0.152	30	7	0.261
	1.8			0.9			0.71			144			45			26		
IC vs IO	1.9	0.4	0.603	0.9	0.2	0.07	0.71	0.1	0.812	132	28	0.496	40	8	0.521	30	9	0.531
	1.8			0.8			0.70			138			39			28		
Shade types																		
FS vs S	1.8	0.8	0.265	0.9	0.1	0.229	0.66	0.2	0.2	124	27	0.165	39	6	0.195	24	11	0.114
	1.9			0.9			0.72			137			42			28		
E vs FS	2.4	0.3	**0.001**	1.2	0.2	**<.001**	0.90	0.1	**<.001**	169	13	**<.001**	51	7	**<.001**	34	5	**0.006**
	1.8			0.9			0.66			124			39			24		
Ser. vs Tim.	2.3	0.8	**<.001**	1.1	0.1	**0.002**	0.90	0.2	**<.001**	162	27	**<.001**	47	6	**0.024**	30	11	0.597
	1.7			0.9			0.64			128			41			28		
LT vs NLT	1.8	0.3	0.614	0.9	0.2	0.567	0.67	0.1	0.64	134	13	0.817	42	7	0.276	33	5	0.057
	1.7			0.9			0.64			137			39			26		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

4.2.4 Impact of fertilizer levels and shade types on the carbohydrates contents of green coffee bean

The disaccharide sucrose content was determined by detecting the hydrolysate of the coffee power using the liquid chromatography-mass spectrometry (LC/MS). Moreover, the polysaccharides contents were decided by measuring the acidolysis solution of coffee power using the high performance liquid chromatography (HPLC). Nonetheless, the monosaccharides contents were not detected due to the undetectable trace amount. The results were shown in the Table 4.6.

There were no significant differences ($P<0.05$) detected in all the carbohydrates contents between the different managements. Furthermore, in terms of shade types, the contents of galactose, mannose, glucose and arabinose were significantly higher ($P<0.05$) under the *Erythrina* system (222 g/Kg, 125 g/Kg, 70 g/Kg, 37.1 g/Kg) than under the full sun system (181 g/Kg, 101 g/Kg, 55 g/Kg, 32.7 g/Kg), while no significant differences ($P<0.05$) were found in other three contrast groups.

Table 4.6 Contrast results for carbohydrates contents (g/Kg) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Sucrose (g/Kg)			Galactose (g/Kg)			Mannose (g/Kg)			Glucose (g/Kg)			Arabinose (g/Kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	92	9	0.544	219	37	0.844	109	17	0.892	69	9	0.828	33.9	4	0.945
	90			217			108			69			33.8		
MC vs IO	88	11	0.053	215	45	0.779	107	21	0.41	67	12	0.525	33.7	5	0.947
	92			217			111			69			33.8		
IO vs LO	87	7	0.915	212	26	0.622	103	12	0.47	67	7	0.899	33.9	3	0.867
	87			218			108			66			34.1		
IC vs IO	90	8	0.328	223	32	0.769	111	15	0.389	70	8	0.689	34.4	3	0.983
	87			220			107			69			34.3		
Shade types															
FS vs S	92	16	0.647	181	64	0.287	101	29	0.037	55	16	0.08	32.7	7	0.173
	90			191			111			65			34.2		
E vs FS	90	7	0.68	222	26	**0.002**	125	12	**<.001**	70	7	**<.001**	37.1	3	**0.002**
	92			181			101			55			32.7		
Ser. vs Tim.	88	16	0.984	231	64	0.222	114	29	0.332	74	16	0.053	35.5	7	0.132
	89			218			109			69			33.8		
LT vs NLT	84	7	0.077	217	26	0.507	109	12	0.599	68	7	0.357	33.9	3	0.701
	90			226			113			71			34.4		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

4.2.5 Impact of fertilizer levels and shade types on the total protein and caffeine contents of green coffee bean

The results of total protein and caffeine contents were shown in Table 4.7. The total protein and caffeine content were significantly lower ($P<0.05$) under the moderate conventional (MC) (153 g/Kg, 11.3 g/Kg) than under the intensive conventional (IC) (168 g/Kg, 12.2 g/Kg). Furthermore, the total protein and caffeine content were significantly higher ($P<0.05$) under the intensive organic (IO) (167 g/Kg, 12.4 g/Kg) than under the moderate conventional (MC) (157 g/Kg, 11.8 g/Kg). Nevertheless, no significant differences ($P<0.05$) were detected in the total protein and caffeine contents between the intensive organic (IO) and low organic (LO), and the intensive conventional (IC) and intensive organic (IO).

For the different shade types, the total protein content of green coffee beans was significantly higher ($P<0.05$) under the full sun system (172 g/Kg) than under the shaded system (159 g/Kg) and the *Erythrina* system (155 g/Kg). In addition, the total protein content of green coffee beans was significantly higher ($P<0.05$) under the service system (165 g/Kg) than under the timber system (146 g/Kg), while it was significantly lower ($P<0.05$) under the non-legume timber system (152 g/Kg) than under the legume timber system (170 g/Kg). The caffeine content of green coffee beans was significantly higher ($P<0.05$) under the full sun system (12.0 g/Kg) than under the shaded system (11.1 g/Kg), whilst no significant differences ($P<0.05$) were detected in the caffeine content between other shade types.

Table 4.7 Contrast results for total protein and caffeine contents (g/Kg) by dry weight of green coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast	Total protein (g/Kg)			Caffeine (g/Kg)		
Managements	Mean	$S.E._D$	p-value	Mean	$S.E._D$	p-value
IC vs MC	168	17	**0.001**	12.2	1.1	**0.003**
	153			11.3		
MC vs IO	157	21	**0.01**	11.8	1.4	**0.01**
	167			12.4		
IO vs LO	162	12	0.94	12.3	0.8	0.681
	162			12.1		
IC vs IO	167	15	0.414	12.4	1.0	0.802
	163			12.3		
Shade types						
FS vs S	172	30	**0.011**	12.0	2.0	**0.014**
	159			11.1		
E vs FS	155	12	**<.001**	11.7	0.8	0.539
	172			12.0		
Ser. vs Tim.	165	30	**<.001**	11.7	2.0	0.405
	146			12.0		
LT vs NLT	170	12	**<.001**	12.2	0.8	0.134
	152			11.5		

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber

4.3 Discussions

4.3.1 How fertilizer levels and shade types affected the physical attributes of green coffee bean

The physical attributes of green coffee bean including density, bulk density, moisture content and roasting loss were investigated in this study. Compared with the full sun system, the major impacts of the shaded system on coffee physiology are related to the reduced wind speeds and temperature fluctuation, which led to the increase of air relative humidity and variations in aerodynamic roughness of the coffee plants. Taken together, these changes decrease leaf-to-air vapour pressure deficit and restrain the stomatal opening, which result in the decline of transpiration rates. Therefore, water loss caused by exceeding coffee tree evapotranspiration will descend (Maestri et al., 2001). In addition, the shaded agroforestry system improves the water status of the coffee tree which is known to affect bean size and density (DaMatta et al., 2007; Elevitch et al., 2014).

The size of ripe cherries and coffee beans increase in the shaded system because the shading decreases air and plant surface temperature and thus prolongs bean ripening to generate the larger fruits (DaMatta et al., 2007).

Therefore, according to the above explanations, in this study, the moisture content of green coffee bean under the full sun system was significantly lower than in the shaded and *Erythrina* system. In terms of service and timber group, the moisture content of green coffee bean under the timber system was significantly higher than in the service system, as the shade cover of the timber shade trees was significantly higher than under the service trees (see Table 3.1

in the Chapter 3).

The density of green coffee bean is around 1300-1400 Kg/m^3 (Sivetz and Desrosier, 1979; Clarke and Macrae, 1988; Dutra et al., 2001) which is higher than the water density (1000 Kg/m^3). As a result, the green coffee bean density would increase with the decreasing moisture content. Therefore, the density of green coffee bean was significantly higher ($P<0.05$) under the full sun system than in the shaded and *Erythrina* systems. However, for service and timber group, no significant difference was detected in green coffee bean density, although the moisture content of green coffee beans was significantly lower under the service system than under the timber system. This may because the shading also prolongs coffee bean ripening to promote the coffee plant to absorb more nutrients. Under the similar shaded system, on the one hand, shading increase the moisture content of green coffee beans, which leads to the decrease of coffee bean density; on the other hand, shading improves the absorbance of nutrients, which may results in the increase of coffee bean density (Cannell, 1974). Under the similar shaded system, these two factors compete with each other during the coffee plant growing, which leads to the result that the density of green coffee bean was no significant difference between the service and timber system.

During the coffee bean roasting process, even though dry matter loss is around 5-8%, the total roasting loss is inconceivably around 15-18% (Eggers and Pietsch, 2001). Furthermore, the water content descends from around 10-12% to 2-3%, when bean temperatures is below 160°C (Gloess, 2014). These demonstrated that the water loss contributed the main mass loss during coffee

bean roasting. As a result, the roasting loss of green coffee bean under the full sun system was significantly lower than in the shaded and *Erythrina* systems due to presenting the lower moisture content in full sun system. Similarly, it can be observed that the roasting loss of green coffee bean under the service system was significantly lower than under the timber system.

4.3.2 How fertilizer levels and shade types affected the mineral content of green coffee bean

As is well known, the ability of the plant to absorb minerals and other nutrients from the soil is extremely complicated. It is determined by the gene as well as the growing environment of plant such as temperature, light intensity, fertilizer level, moisture content and salinity of soil. (Martínez-Ballesta et al., 2010).

In our experiment, the greater quantities of potassium (K) and phosphorus (P) in green coffee beans under the intensive organic (IO) management compared to the intensive (IC) and moderate conventional (MC) managements were related to the higher input of these elements in the intensive organic (IO) management (see Table 2.4 in the Chapter 2). However, in terms of intensive organic (IO) and low organic (LO) managements, although the application amounts of potassium (K) and phosphorus (P) in the intensive organic (IO) (326 kg/ha/year, 205 kg/ha/year) were much higher than in the low organic (LO) management, which was 44 kg/ha/year and 2 kg/ha/year for K and P, respectively, no significant differences in potassium (K) and phosphorus (P) contents of green coffee beans were detected between these two managements. Haggar et al. (2011) reported that the organic management improved the organic matters content and cation exchange capacity of soil in Costa Rica site.

Therefore, although the application amounts of potassium (K) and phosphorus (P) in the low organic (LO) management were much lower, the low organic (LO) management was able to make up for the low of input amounts by more organic matter and a stronger cation exchange capacity.

The higher calcium (Ca) content of green coffee bean in the intensive organic (IO) management was connected with the high content of calcium (Ca) in the chicken manure (Boateng et al., 2006). In our experiment, the chicken manure was used as the fertilizer in the intensive organic (IO) management. Additionally, the contents of manganese (Mn), copper (Cu) and zinc (Zn) of green coffee beans were significantly lower ($P<0.05$) in the intensive organic (IO), while the molybdenum (Mo) content was significantly higher ($P<0.05$) in the intensive organic (IO) management, as organic management decreased the soil acidity or increased soil pH (Haggar et al., 2011), and, thus, restricted the absorptivity of manganese (Mn), copper (Cu) and zinc (Zn) (Sims, 1986) and improved the absorbance of molybdenum (Mo) (Olsen and Watanabe, 1979). For sodium (Na), its content was significantly lower ($P<0.05$) in the intensive organic (IO) than in the moderate conventional (MC) and low organic (LO), as the higher absorbance of calcium (Ca) in the intensive organic (IO) restrained the sodium (Na) uptake (Lynch and Läuchli, 1984).

The moisture content of soil, light intensity and temperature are the main external factors to affect the absorbance and transportation of minerals in the plant (Tromp, 1975; Caruso et al., 2003; Hu and Schmidhalter, 2005, Montanaro et al., 2006). The Costa Rica site is considered as a wet coffee zone with an average annual rainfall of 2,915 mm/year. Thus, all coffee plants and

corresponding soils in different shade types have no shortage of water and the moisture content cannot be considered as a factor to affect the mineral absorption of coffee plant from soil. As mentioned in 4.3.1, shade system reduces the temperature and light intensity of coffee plant as well as soil (DaMatta et al., 2007). Nonetheless, the ability of plant to absorb minerals increases with the increasing temperature when temperature is below 40°C (Starck et al., 1994). Moreover, the higher light intensity improves the absorptivity of minerals in plant (Caruso et al., 2003). Therefore, the contents of sulfur (S), calcium (Ca) and manganese (Mn) were significantly higher ($P<0.05$) in the full sun system than in the shaded and *Erythrina* systems due to the higher temperature and light intensity. However, the contents of copper (Cu), zinc (Zn) and sodium (Na) were significantly lower ($P<0.05$) in the full sun system than in the shaded and *Erythrina* systems due to the antagonism of calcium (Ca) and copper (Cu), zinc (Zn) and sodium (Na) (Lynch and Läuchli, 1984). Compared with copper (Cu), zinc (Zn) and sodium (Na) uptakes, the coffee plant absorbed much more calcium (Ca) (see Table 4.2 and 4.3), therefore, the excess of calcium (Ca) uptake restricted the coffee plant to uptake more copper (Cu), zinc (Zn) and sodium (Na).

4.3.3 How fertilizer levels and shade types affected the total lipid and fatty acid content of green coffee bean

The formation of lipid in coffee plant is a complicated progress, which includes many complex biochemical reactions especially the enzymatic reactions. Furthermore, the energy and some chemical elements such as carbon, hydrogen, oxygen, nitrogen and phosphorus play an essential role in the lipid biosynthesis

in coffee plant (Ohlrogge and Browse, 1995). Ohlrogge and Browse (1995) stated that the acetyl-coenzyme A (CoA) in the plastid contributed all the carbon atoms present in the fatty acids, and they also explained all possible lipid biosynthesis pathways in the plant. In addition, they considered that the synthesis rate of fatty acid in leaves was six times higher in the light than in the dark. As a result, in our experiment, different fertilizer levels and shade types (light intensity) might affect the total lipid and fatty acids contents in the green coffee beans.

In terms of different fertilizer levels, the total lipid content was significantly lower ($P<0.05$) under the moderate conventional (MC) than under the intensive conventional (IC) management due to the lower input amount of fertilizer in the moderate conventional (MC) management (see Table 2.4 in the Chapter 2). Although the slower release of plant available nutrients and lower availability of organic fertilizers in the intensive organic (IO) management with even a higher fertilizer input level (see Table 2.4 in the Chapter 2) (Castro-Tanzi et al., 2012), the total lipid and all fatty acids contents under the intensive organic (IO) were similar to those under the intensive conventional (IC) management and even significantly higher ($P<0.05$) for the total lipid and a few fatty acids such as palmitic acid, oleic acid and cosenoic acid than under the moderate conventional (MC) management after a long period about 13 years (the green coffee bean samples used in this study were harvested in 2013 and 2014). Therefore, in the long term, in terms of the amount of total lipid and fatty acids in the green coffee beans, the performance of intensive organic (IO) management with a higher fertilizer input amount kept up with its counterpart intensive conventional (IC) and to some extent, even exceeded the moderate

conventional (MC) management, as the intensive organic (IO) management improved soil environment and increased cation exchange capacity and organic matters in soil (Haggar et al., 2011). Furthermore, for the intensive organic (IO) and low organic (LO), although the input amount of fertilizer in the low organic (LO) was much lower, no significant difference was detected in the total lipid and fatty acids contents between these two organic managements. In our contrast model (see Table 2.5 in the Chapter 2), when compared with the intensive organic (IO), the low organic (LO) management was combined with the legume species *Erythrina* and *Chloroleucon*. On the one hand, the legume species can replenish the lack of N_2 due to the low input level by N_2 fixation (Nygren et al., 2012); on the other hand, the high pruning intensity like *Erythrina* may compensate the lower external inputs, as the leaves and branches pruned from the *Erythrina* shade tree were left on the field (Haggar et al., 2011).

With regards to the effect of shade types on the total lipid and fatty acids contents, there were no significant differences detected between the full sun and shaded system, and the legume timber and non-legume timber system, which proved that the light intensity and N_2 fixation were not the main factors to affect the lipid content in our experiment. Nonetheless, the total lipid and fatty acids contents were significantly higher ($P<0.05$) under the *Erythrina* system than under the full sun and timber systems (the *Erythrina* was the only service shade tree in our experiment, thus, in the contrast group service vs timber, service tree could be replaced directly by *Erythrina*). It could be explained by the intensive pruning of *Erythrina* (Boreux et al., 2016). The intensive pruning of *Erythrina* supplied available nutrients directly to the soil

as well as to the coffee plant, which promoted the biosynthesis of lipid in the coffee plant.

4.3.4 How fertilizer levels and shade types affected the carbohydrates contents of green coffee bean

It is well known that the inorganic carbon is converted to the organic carbon in the plant mainly through photosynthesis, and also, the plant can also absorb the organic carbon fixed by fungi as well as from organic matters on the field (Kucey and Paul, 1981; Stumpf et al., 2012). In addition, photosynthesis is affected by light intensity, carbon dioxide concentration, water availability, temperature. (Boardman, 1977; Robarts and Zohary, 1987). For each contrast group of different managements in our experiment, the shade types have been fixed (see Table 2.5 in Chapter 2). Thus, it could be assumed that light intensity and temperature were the same when comparing the different managements in each contrast group. Different managements had no effect on the carbon dioxide concentration due to the same atmospheric conditions. Furthermore, Costa Rica site was considered as the wet coffee zone, which had the adequate water resource. Therefore, no significant differences ($P<0.05$) were detected in the carbohydrates contents between the different managements.

In our experiment, there were no significant differences ($P<0.05$) found in the carbohydrates contents between the full sun and shaded system, service and timber, and the legume timber and non-legume timber system, which demonstrated that the light intensity, shaded cover and the N_2 fixation of shade tree were not the main factors to affect the carbohydrates contents of green coffee beans. Nevertheless, the galactose, mannose, glucose and arabinose

contents were significantly higher ($P<0.05$) under the *Erythrina* system when compared to full sun system. This was related to the more organic matter especially the organic carbon used directly by the coffee plant due to the extreme pruning of the *Erythrina* shade tree (the branches and leaves of *Erythrina* were maintained on the field after pruning) (Boreux et al., 2016).

4.3.5 How fertilizer levels and shade types affected the total protein and caffeine contents of green coffee bean

The total protein and caffeine contents were significantly higher ($P<0.05$) under the intensive conventional (IC) and intensive organic (IO) managements than under the moderate conventional (MC) due to the higher input amount of fertilizers especially the nitrogen. After a long period management (about 13 years), the intensive organic (IO) management had a similar total protein and caffeine contents in the green coffee beans to the intensive conventional (IC) management and even a significantly higher ($P<0.05$) value than the moderate conventional (MC) management. Nonetheless, there was no significant difference ($P<0.05$) in the total protein and caffeine contents between the intensive organic (IO) and low organic (LO) system. When compared with the intensive organic (IO) management, the low organic (LO) management was combined with the legume species *Erythrina* and *Chloroleucon* (see Table 2.5 in the Chapter 2). On the one hand, the legume species can replenish the lack of N_2 due to the low input level by N_2 fixation (Nygren et al., 2012); on the other hand, the high pruning intensity like *Erythrina* may compensate the lower external inputs, as the leaves and branches pruned from the *Erythrina* shade tree were left on the field (Haggar et al., 2011, Boreux et al., 2016).

The result of the effect of different shade types on the total protein content suggested that light intensity was the main factor to drive the protein generation in the green coffee beans in our experiment, as the protein biosynthesis in the ribosome is adenosine triphosphate (ATP) dependent (Boulter. 1970). As a result, the total protein content was significantly higher ($P<0.05$) under the full sun system than under the shaded and *Erythrina* system due to the higher shade cover (see Fig. 3.3 in the Chapter 3) and lower light intensity in the shaded and *Erythrina* systems, moreover, it was also significantly higher ($P<0.05$) under the service system than under the timber system due to the lower shade cover (see Table 3.1 in the Chapter 3) and higher light intensity in the service system (the *Erythrina* was the only service tree in our experiment). Additionally, the total protein content in the green coffee beans was significantly higher ($P<0.05$) under the legume timber system than under the non-legume timber system due to the N_2 fixation of the legume species to supply the organic N_2 for the coffee plant to synthesize the amino acids and protein. With regards to the caffeine content in the green coffee beans, it was significantly higher ($P<0.05$) under the full sun system than under the shaded system, as the light improved the formation of the purine ring of caffeine (Anderson and Gibbs, 1962). Nevertheless, the caffeine content in the green coffee beans was no significant difference ($P<0.05$) between the legume timber and non-legume timber system, even though the legume trees could fix the N_2 which is the main component of caffeine. Furthermore, there were no significant differences ($P<0.05$) in the caffeine contents of green coffee beans between the *Erythrina* and the full sun system, and service and timber system, although the shade cover affecting the light intensity between these shade

systems was significantly different (see Table 3.1 and Fig. 3.3 in the Chapter 3). As a result, the N_2 fixation in the legume tree and the differences of shade cover between the *Erythrina* and the full sun system, and the service and timber system were not the main factors to affect the caffeine content in the green coffee beans.

4.4 Conclusions

To conclude, the different cultivation managements or fertilizer input amounts (N:P:K) had no significant effects on the physical attributes such as density, bulk density, moisture content and roasting loss and the carbohydrate content of green coffee beans, while they had significant impacts on the minerals, total lipid, fatty acids, total protein and caffeine contents. The contents of potassium (K), phosphorus (P) and calcium (Ca) were significantly higher ($P<0.05$) under the intensive organic (IO) management due to a higher input amounts of fertilizer (N:P:K) and calcium (Ca) content in the chicken manure. The effect of different managements on the other minerals was attributed to the impact of organic input on the soil conditions such as pH and cation exchange capacity as well as the competition between these mineral ions. The different input amounts of fertilizers (N:P:K) could affected the total lipid and a few fatty acids contents, total protein and caffeine contents.

The different shade types could affect the physical attributes of green coffee beans such as bean density, moisture content and roasting loss due to the variation of microclimate of the coffee plant under the different shade trees. The differences of mineral contents in the different shade types were related to the differences of light intensity and temperature in different shade managements as well as the competition between these mineral ions. Moreover, significantly higher ($P<0.05$) total lipid, fatty acids and polysaccharides contents of green coffee beans were found under the *Erythrina* system due to the extremely strong pruning of *Erythrina* shade tree and the recycling of its branches and leaves on the field. The differences of light intensity and N_2

fixation ability caused by the different shade types had no significant effect ($P<0.05$) on the contents of total lipid, fatty acids and carbohydrates. Nevertheless, the differences of light intensity in the different shade managements had a significant effect ($P<0.05$) on the total protein and caffeine contents, and the legume tree species could improve the total protein amount in the green coffee beans by fixing more N_2.

This chapter (Chapter 4) investigated and discussed the effect of different fertilizer levels and shade types of coffee plants on the physical and chemical attributes of green coffee beans, and the next chapter (Chapter 5) explores the relationship between the different fertilizer levels and shade types of coffee plants and volatile compound generation during roasting of green coffee beans, through studying the relationship between the physical and chemical attributes of green coffee beans in different agricultural treatments and volatile compounds generation in the roasted coffee beans.

Chapter 5 Impact of fertilizer levels and shade types of coffee plants on the aroma generation of roasted coffee beans

5.1 Introduction

Coffee is widely appreciated and consumed for its pleasant aroma and taste generated during the roasting process (Fisk et al., 2012). There are many sourcess affecting the aroma generation of roasted coffee beans: the origin of coffee beans (i.e. Arabica, Robusta) may contribute to the differences (Semmelroch and Grosch, 1996); seasonal variation may also play an essential role in differences (Agresti et al., 2008; Silva et al., 2005); additionally, differences in geographical locations (Risticevic et al., 2008), differences in post-harvest processing (wet or dry processing) and ageing before roasting also affect the final aroma generation (Fisk et al., 2012). Furthermore, roasting profile (time-temperature) and the roaster models are aslo significant contributors to the differences of final coffee flavour (Franca et al., 2009; Bhumiratana et al., 2011).

It is well known that green coffee beans are largely non-aromatic and contain green and peasy notes (Holscher and Steinhart, 1995). However, the green coffee beans contain large number of chemical precursors such as sucrose, organic acids, proteins, lipids that contribute significantly to the formation of coffee aroma and taste (Tressl, 1989; Holscher and Steinhart, 1994; Ho et al, 1993; Reineccius, 1995). The amounts of these precursors significantly vary between coffees due to their origin and treatments. During roasting a large number of different chemical reactions (Maillard reactions, caramelisation, lipid oxidation) occur to generate a complex mixture of aroma compounds

(Fisk et al., 2012).

Over 850 aroma compounds have been found in roasted coffee, these include sulphur compounds, pyrazines, pyridines, pyrroles, oxazoles, furans, aldehydes, ketones, phenols and other heterocyclic compounds (Grosch, 2001). Identification and quantification of coffee volatile aroma compounds are regarded as a challenge due to the wide range of concentrations, high volatilities, wide range of physicochemical attributes and their potential to polymerise and bind to other coffee components (Fisk et al., 2012).

Gas chromatograph is widely used to analyse the coffee volatile composition followed by mass spectrometer (Yeretzian et al., 2003) or other specific detectors (flame ionization detectors (Freitas and Mosca, 1999), nitrogen-phosphorous detectors, photo-ionization detectors) due to its sensitivity and operability. It can separate and identify a complex mixture of aromas in one operation as well as quantify the volatiles at extremely low concentrations (Dart and Nursten, 1985).

In the Chapter 4, the effects of agro-forestry systems including fertilizer levels (organic and non-organic) and shade types on the physical and chemical attributes of green coffee beans has been examined. We have proved that the levels and types of fertilizer and the species of shade trees had a significant effect on the chemical composition of green coffee beans. As a result, it can be assumed that the growth conditions of coffee plants could directly affect the generation of aroma of roasted coffee beans through differences in the chemical composition of the green coffee beans. The aim of this chapter was to explain this interaction and detail the specific effects of fertilizer levels and

shade types on aroma generation of roasted coffee beans.

5.2 Results

5.2.1 Aroma compounds of roasted coffee beans in the different agricultural treatments

Sixty-four volatile aroma compounds, which have previously been identified in coffee, were detected in the roasted coffee beans. These aroma compounds were classified into thirteen groups based on their chemical attributes (3 alcohols, 4 aldehydes, 11 ketones, 3 acids, 1 ether, 2 esters, 14 pyrazines, 2 pyridines, 7 pyrroles, 2 sulphides, 8 furans, 5 phenolic compounds and 2 N-containing heterocyclic compounds), and their odours and functional group were described (Table 5.1). Fig 5.1 showed the gas chromatogram of roasted coffee beans and identified some main peaks of volatile aroma compounds based on the library (NIST/EPA/NIH Mass Spectral Library. Version 2.0, Faircom Corporation, U.S.). The quantitative data sets of the volatile aroma compounds measured in the current work can be found in Appendix I (pages 145 to 157).

Table 5.1 Detection of sixty-four volatile aroma compounds in the roasted coffee beans in the different agricultural treatments

	Retention time	Aroma compound	Odour description	Functional group
1	2.14	2-Methylfuran	Chocolate	Furan
2	2.40	2-Methylbutanal	Malty	Aldehyde
3	2.42	3-Methylbutanal	Malty	Aldehyde
4	2.75	2,5-Dimethylfuran	Ethereal	Furan

5	3.04	2,3-Butanedione	Buttery, cheesy	Ketone
6	4.24	2,3-Pentanedione	Oily buttery	Ketone
7	4.43	Dimethyl-Disulphide	Onion	Sulphide
8	4.46	2-Vinylfuran	Ethereal, rum, cocoa note	Furan
9	4.55	Hexanal	Grassy, green oily	Aldehyde
10	5.60	2,3-Hexanedione	Buttery, cheesy, sweet, creamy	Ketone
11	5.70	1-Methylpyrrole	White bread, woody	Pyrrole
12	6.04	p-Cresol	Phenolic-type	Phenolic
13	6.55	Pyridine	Bitter, astringent, roasted, burnt	Pyridine
14	6.92	2,4,5-Trimethyloxazole	Green, woody, musty	Heterocyclic N
15	8.10	2-Pentylfuran	Fruity, green	Fruan
16	8.11	Furfuryl methyl ether	Nutty, rich, phenolic	Ether
17	8.65	2-Methylpyrazine	Nutty, roasted, chocolate	Pyrazine
18	8.67	Dihydro-2-methyl-3-furanone	Sweet, roasted	Ketone
19	9.06	4-Methylthiazole	Nutty, green	Heterocyclic N
20	9.60	Acetoin	Buttery, creamy	Ketone
21	10.09	2,5-Dimethylpyrazine	Nutty, roasted, grassy, corn	Pyrazine
22	10.29	2,6-Dimethylpyrazine	Nutty, sweet, fried	Pyrazine
23	10.41	2-Ethylpyrazine	Nutty, roasted	Pyrazine
24	10.71	2,3-Dimethylpyrazine	Nutty, roasted, green	Pyrazine
25	11.26	2-Methyl-2-cyclopentenone	Fruity	Ketone
26	11.76	2-Ethyl-6-methylpyrazine	Roasted, hazelnut-like	Pyrazine
27	11.90	2-Ethyl-5-methylpyrazine	Nutty, roasted	Pyrazine
28	12.25	2,3,5-Trimethylpyrazine	Nutty, roasted	Pyrazine
29	12.34	2-Ethyl-3-methylpyrazine	Nutty	Pyrazine

30	12.44	Propyl pyrazine	Green	Pyrazine
31	13.04	2,6-Diethylpyrazine	Nutty, roasted	Pyrazine
32	13.19	Vinyl pyrazine	Nutty, green	Pyrazine
33	13.35	2-Ethyl-3,6-dimethylpyrazine	Nutty, roasted	Pyrazine
34	13.75	2-Ethyl-3,5-dimethylpyrazine	Nutty	Pyrazine
35	13.80	Acetic acid	Sour	Acid
36	14.01	Furfural	Bread, almond, sweet	Aldehyde
37	14.17	Acetoxy acetone	Buttery	Ketone
38	14.51	2-Fufurylmethylsulfide	Alliaceous, sulfurous	Sulphide
39	14.99	2-Acetylfuran	Balsamic-sweet	Furan
40	15.32	Pyrrole	Nutty, hay-like, herbaceous	Pyrrole
41	15.72	2,3-Dimethyl-2-cyclopentenone	Grassy, bitter	Ketone
42	15.84	Acetoxy-2-butanone	Sour	Ketone
43	15.93	2-Furfurylacetate	Fruity, green	Ester
44	16.05	Propionic acid	Sour	Acid
45	16.39	3-Methylpyrrole	Woody	Pyrrole
46	16.75	5-Methylfurfural	Sweet, caramel, bready	Furan
47	17.35	2-Acetylpyridine	Popcorn type, corn type	Pyridine
48	17.80	1-Methyl-2-formylpyrrole	Bread, burnt, caramel	Pyrrole
49	17.93	g-Butyrolactone	Creamy, milky	Ester
50	19.00	Furfuryl alcohol	Burnt	Alcohol
51	19.24	Isovaleric acid	Cheesy	Acid
52	19.41	2-Furfuryl-5-methylfuran	Chocolate	Furan
53	20.96	2,5-Dihydrofuranone	Caramel	Ketone
54	22.79	1-Furfurylpyrrole	Vegetable	Pyrrole
55	23.47	Guaiacol	Phenolic, woody	Phenolic

56	24.56	Phenylethyl alcohol	Floral	Alcohol
57	25.28	2-Thiophenemethanol	savory	Alcohol
58	25.85	2-Acetylpyrrole	White bread	Pyrrole
59	26.21	Difurfuryl ether	Coffee-like, toasted odour	Ether
60	26.72	Phenol	Smoky	Phenolic
61	26.94	2-Formylpyrrole	White bread, jasmine rice	Pyrrole
62	27.09	4-Ethylguaiacol	Phenolic	Phenolic
63	27.18	Furaneol	Fruity	Ketone
64	30.42	2-Methoxy-4-vinylguaiacol	Woody, smoky	Phenolic

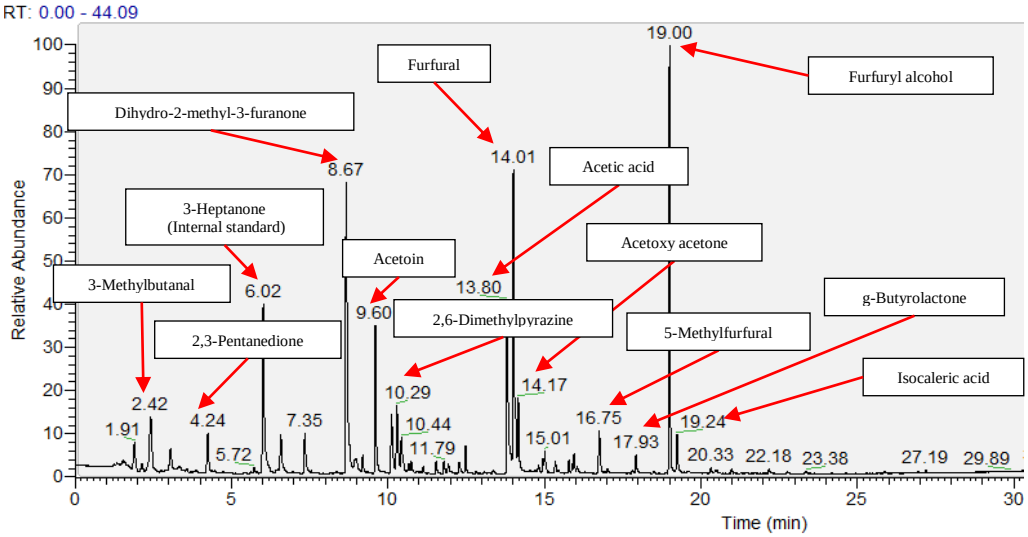


Fig 5.1 Gas chromatogram of head space of roasted coffee powders on ZB-WAX capillary column

5.2.2 Impact of different fertilizer levels on the aroma generation of roasted coffee beans

For each contrast model, the fertilizer level with the relatively lower amount of

volatile compounds generation was assumed as the control group and the other one with the relatively higher amount of volatile compounds generation was supposed to be the non-control group. The values of these volatile compounds in the control group were defined as 100. The ratio of the non-control group to the control group were calculated, and then, the final values of these volatile compounds in the non-control group were expressed as the ratio multiplied by 100. For example, in the Fig. 5.2, the moderate conventional (MC) with the relatively lower volatile compounds generation was assumed as the control group and showed as the circle with round markers at 100%, while the intensive conventional (IC) with the relatively higher abundance of volatile compounds was defined as the non-control group and showed as the spider diagram with diamond markers. Although sixty-four volatile compounds were detected in our experiment, only sixty volatile compounds were displayed in Fig. 5.2-5.9, as there were no significant differences ($P<0.05$) in the amounts of these four volatile compounds (2-Methylbutanal, Isovaleraldehyde, Phenol and Furaneol) between the different fertilizer levels and shade types. Fig. 5.2, 5.3, 5.4 and 5.5 showed the effect of different fertilizer levels on the aroma generation of roasted coffee beans.

The relative abundance of twenty volatile compounds were significantly higher ($P<0.05$) in the roasted coffee beans under the intensive conventional (IC) management than in those roasted coffee beans under the moderate conventional (MC) management (Fig. 5.2). The amounts of twenty-five volatile compounds were significantly higher ($P<0.05$) in the roasted coffee beans under the intensive organic (IO) management than in those roasted coffee beans under the moderate conventional (MC) management (Fig. 5.3).

Nevertheless, only five volatile compounds had significantly higher ($P<0.05$) amounts in the roasted coffee beans under the intensive organic (IO) management than in those roasted coffee beans under the low organic (LO) management (Fig. 5.4). Moreover, there were eight volatile compounds with a significantly higher ($P<0.05$) amount in the roasted coffee beans under the intensive conventional (IC) management than in those roasted coffee beans under the intensive organic (IO) management (Fig. 5.5).

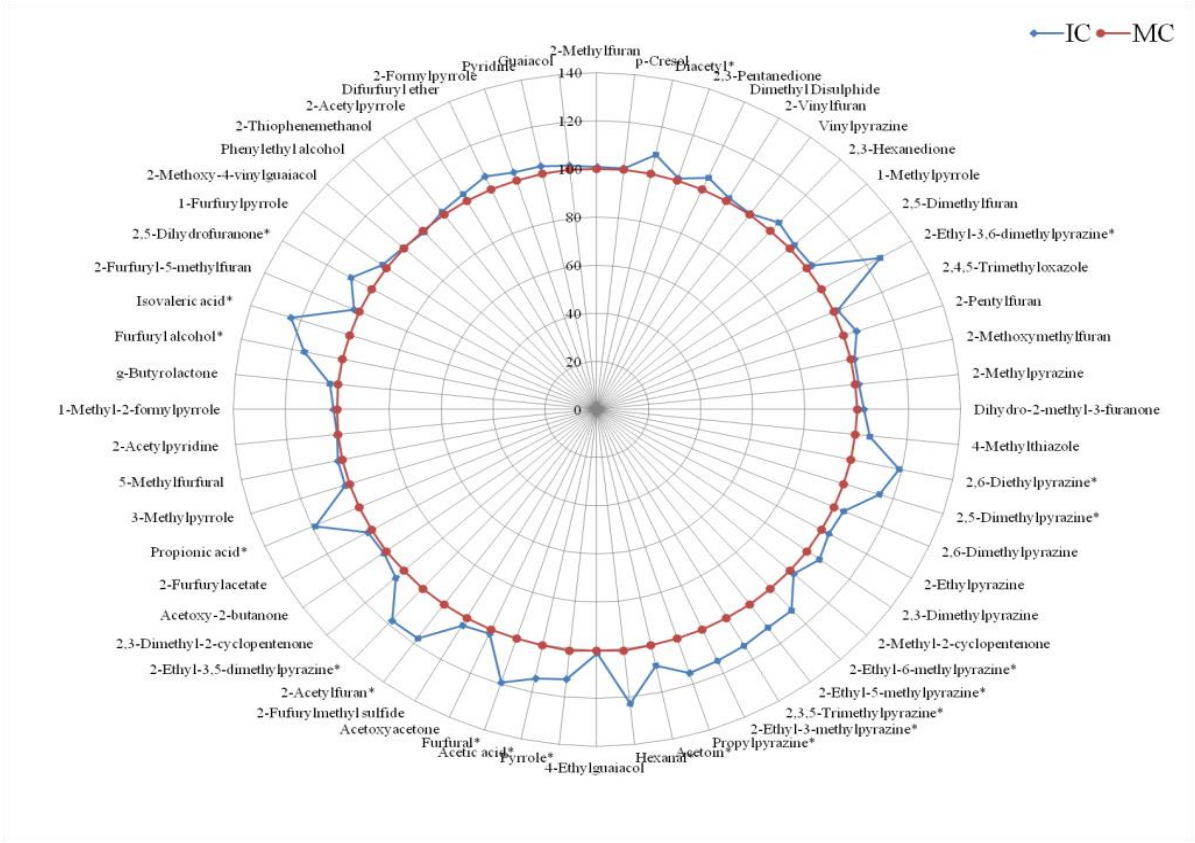


Fig. 5.2 Comparison of volatile aroma compounds generation in roasted coffee beans between the intensive conventional (IC) and moderate conventional (MC) managements, results were presented as the mean value of three replicates.

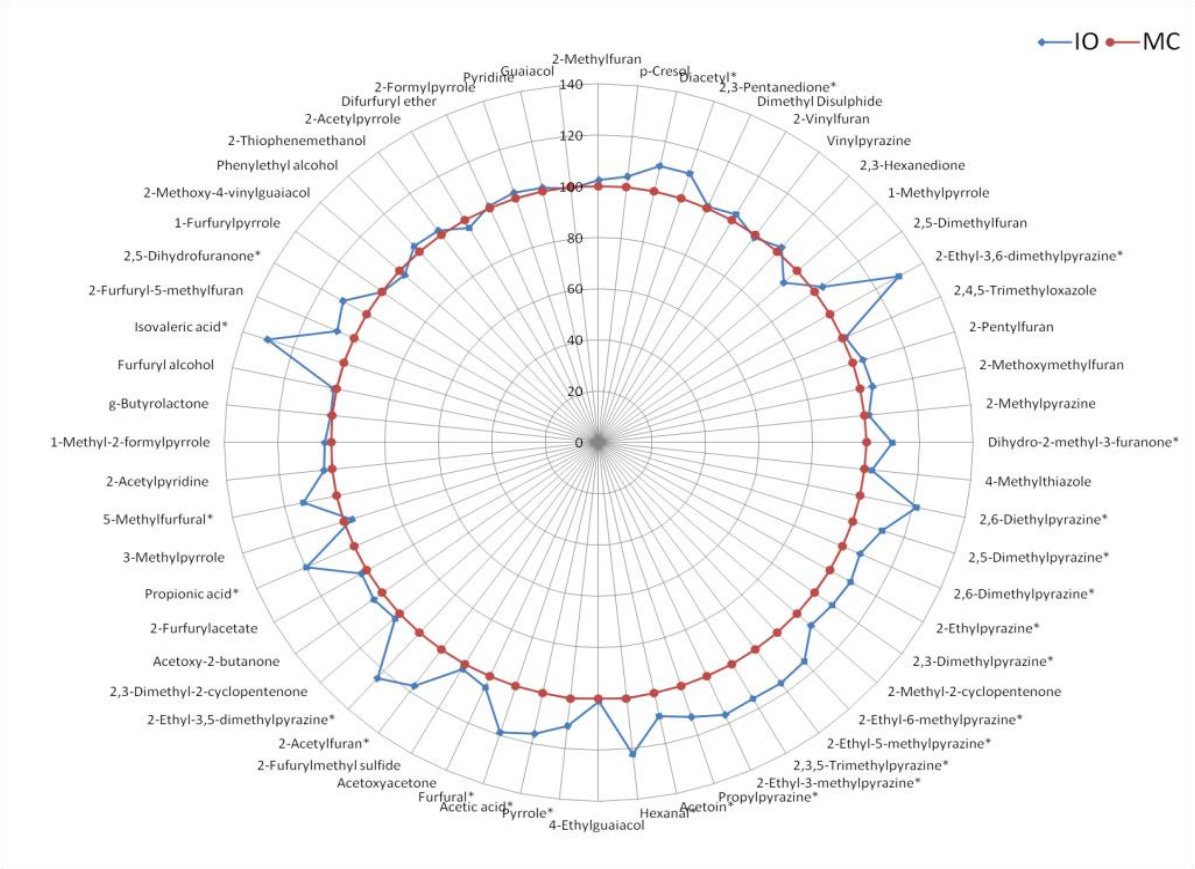


Fig. 5.3 Comparison of volatile aroma compounds generation in roasted coffee beans between the intensive organic (IO) and moderate conventional (MC) managements, results were presented as the mean value of three replicates.

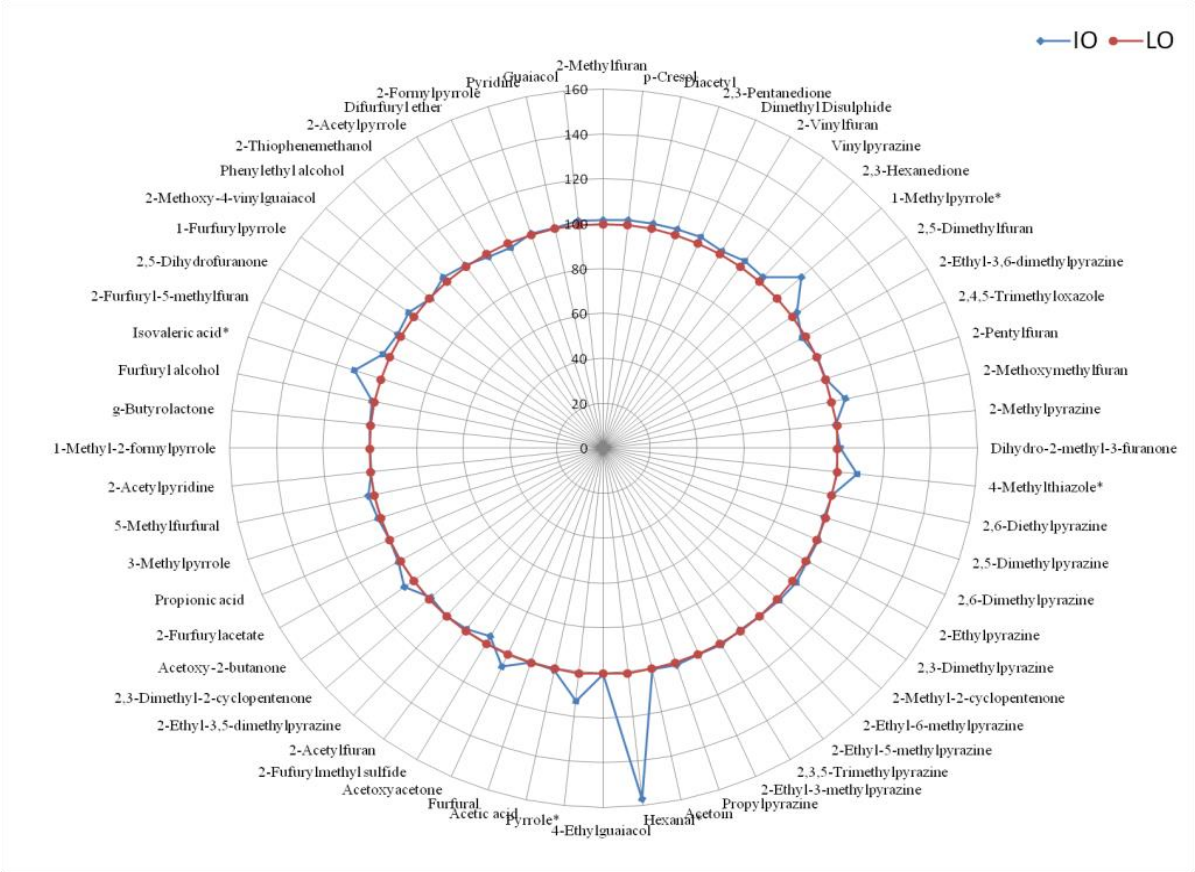


Fig. 5.4 Comparison of volatile aroma compounds generation in roasted coffee beans between the intensive organic (IO) and low organic (LO) managements, results were presented as the mean value of three replicates.

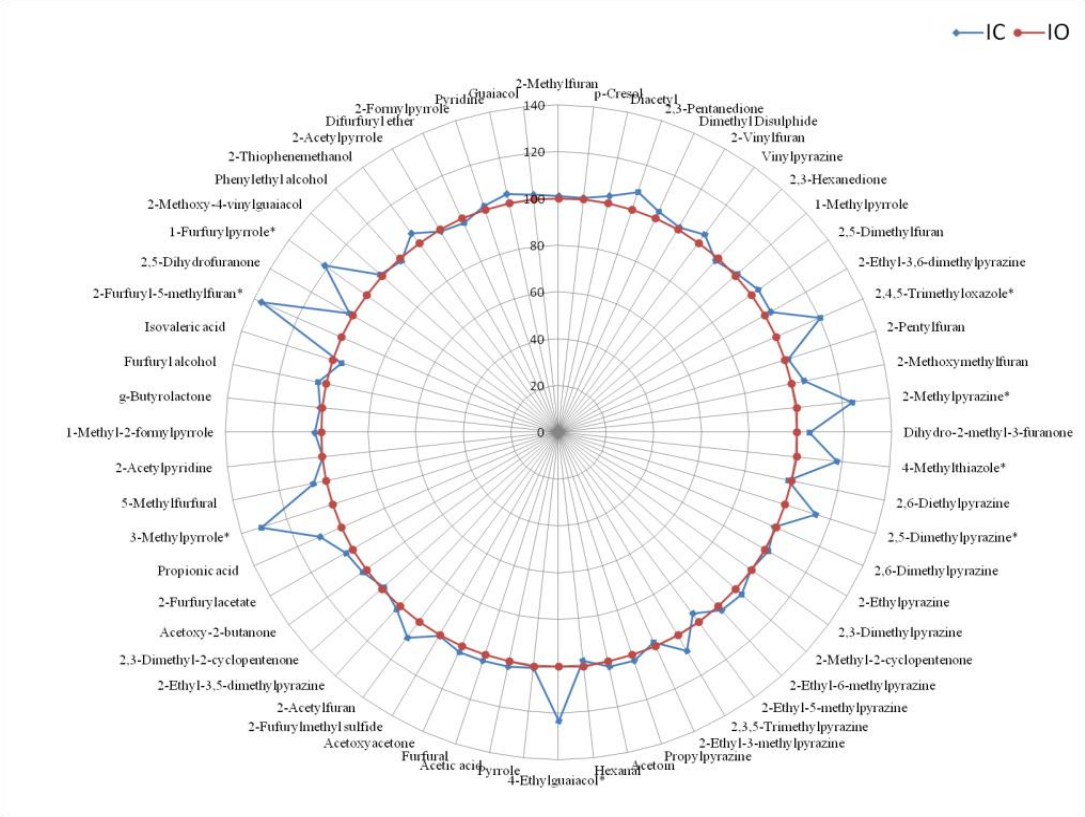


Fig. 5.5 Comparison of volatile aroma compounds generation in roasted coffee beans between the intensive conventional (IC) and intensive organic (IO) managements, results were presented as the mean value of three replicates.

5.2.3 Impact of different shade types on the aroma generation of roasted coffee beans

Similarly, for each contrast model, the shade type with the relatively lower volatile compounds generation was assumed as the control group and the other one with the relatively higher volatile compounds generation was supposed to be the non-control group. The values of these volatile compounds in the control group were defined as 100. The ratio of the non-control group to the control group was calculated, and then, the final values of these volatile compounds in the non-control group were expressed as the ratio multiplied by 100. Fig. 5.6, 5.7, 5.8 and 5.9 showed the effect of different shade types on the aroma generation of roasted coffee beans.

Thirty-six volatile compounds had a significantly higher ($P<0.05$) amounts in the roasted coffee beans under the full sun system than in those roasted coffee beans under the shaded system (Fig. 5.6). The amounts of forty volatile compounds were significantly higher ($P<0.05$) in the roasted coffee beans under the full sun system than in those roasted coffee beans under the *Erythrina* system (Fig. 5.7). Additionally, there were forty-three volatile compounds with a significantly higher ($P<0.05$) amount in the roasted coffee beans under the service shade tree system than in those roasted coffee beans under the timber shade tree system (Fig. 5.8).The amounts of fifty-one volatile compounds were significantly higher ($P<0.05$) in the roasted coffee beans under the legume timber shade tree system than in those roasted coffee beans under the non-legume timber shade tree system (Fig. 5.9).

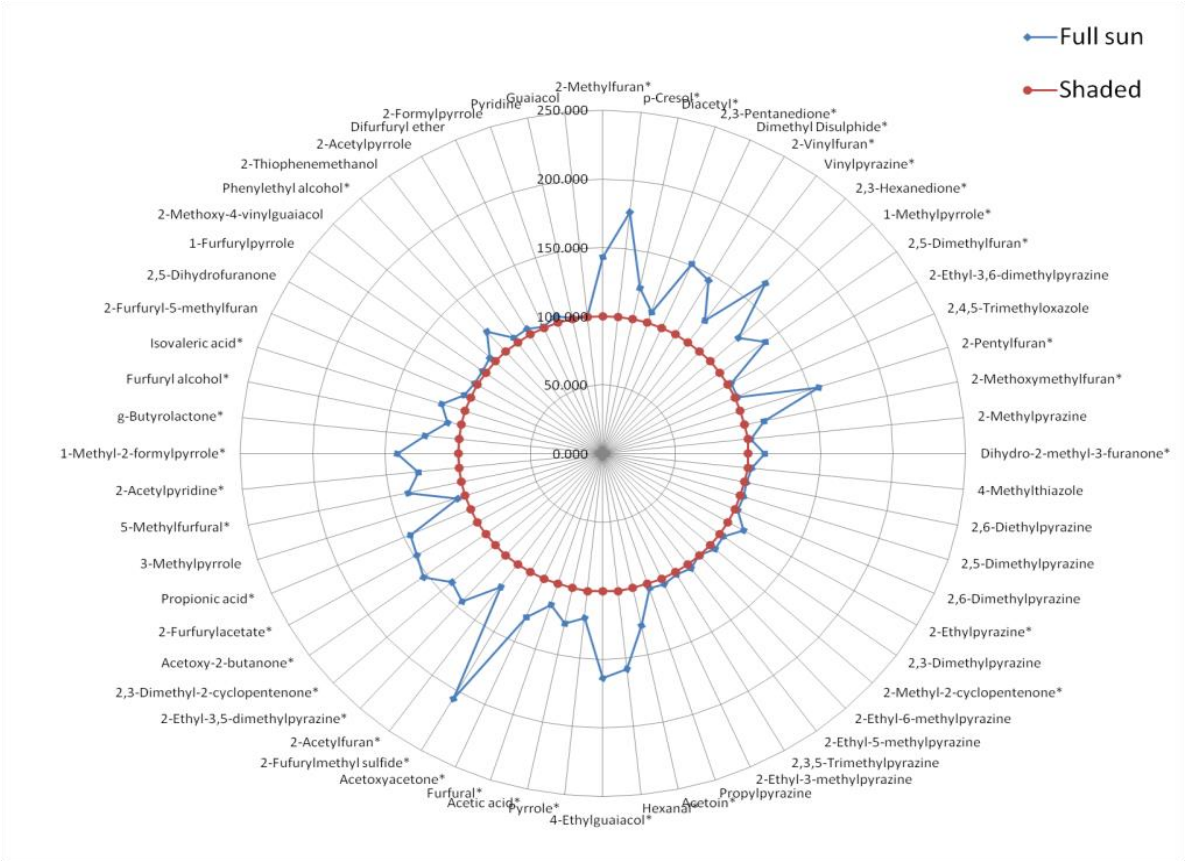


Fig. 5.6 Comparison of volatile aroma compounds generation in roasted coffee beans between the full sun and shaded systems, results were presented as the mean value of three replicates.

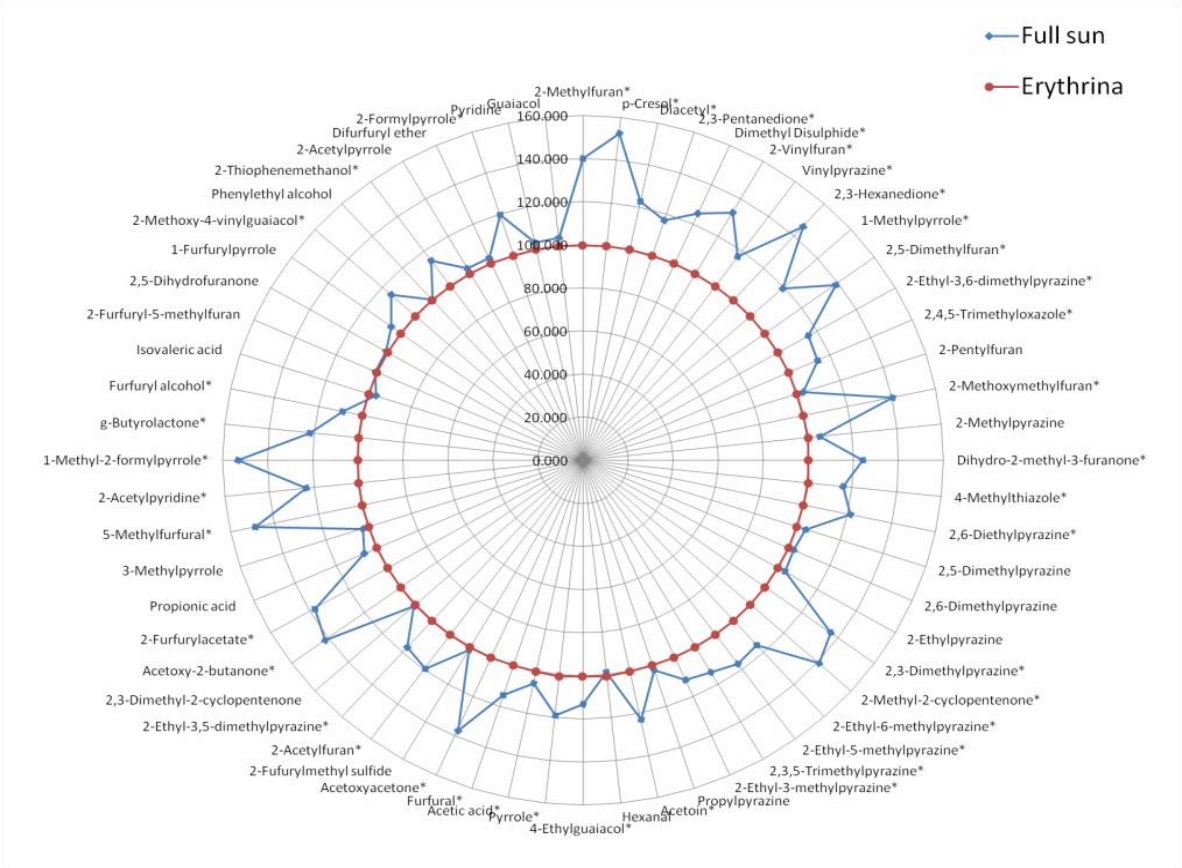


Fig. 5.7 Comparison of volatile aroma compounds generation in roasted coffee beans between the full sun and *Erythrina* systems, results were presented as the mean value of three replicates.

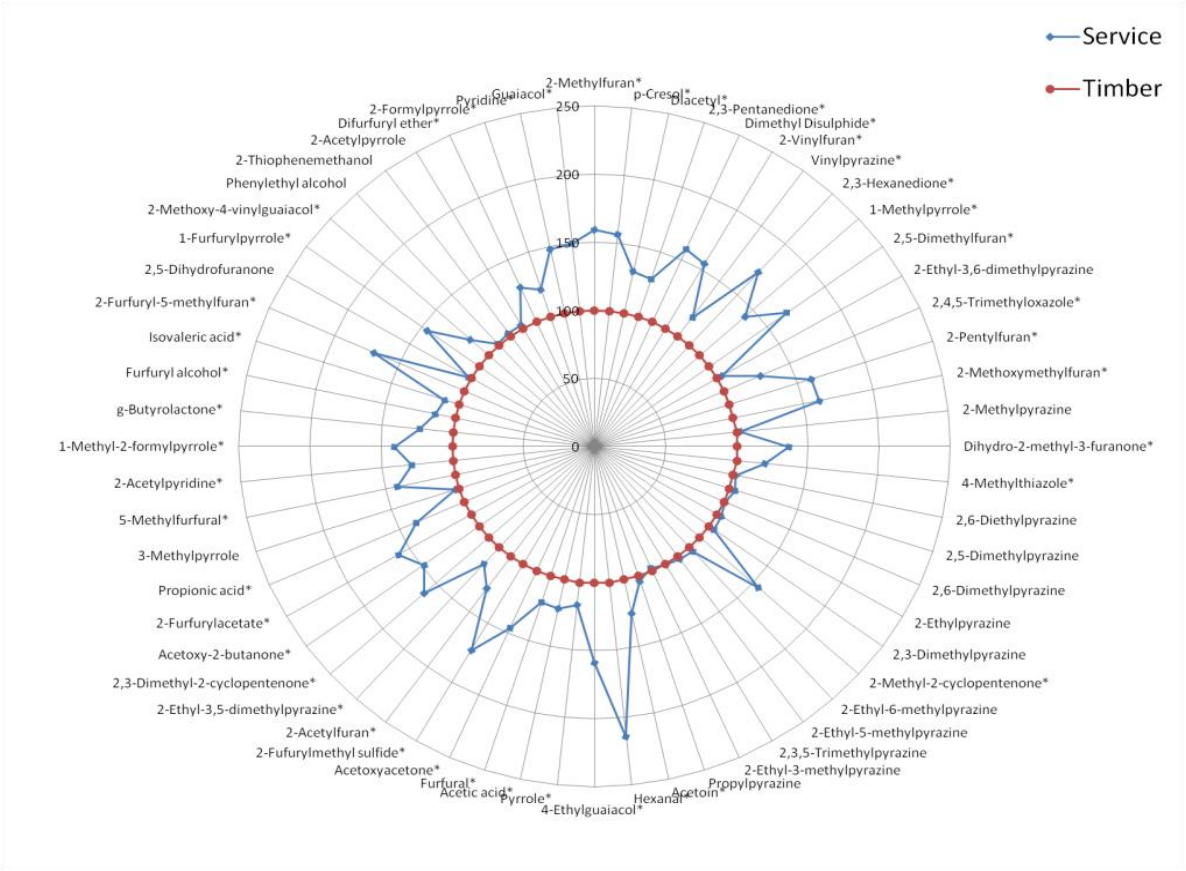


Fig. 5.8 Comparison of volatile aroma compounds generation in roasted coffee beans between the service and timber tree systems, results were presented as the mean value of three replicates.

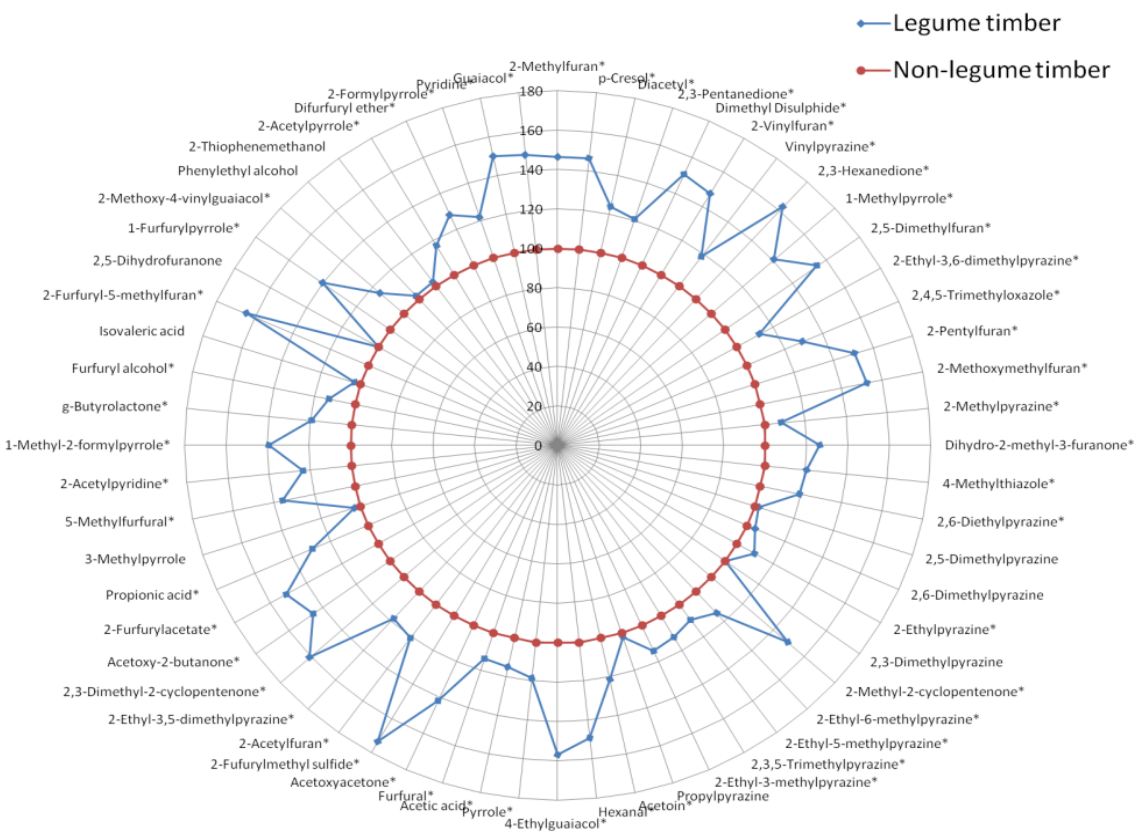


Fig. 5.9 Comparison of volatile aroma compounds generation in roasted coffee beans between the legume timber and non-legume timber tree systems, results were presented as the mean value of three replicates.

5.2.4 Impact of different fertilizers and shade types on the colour of roasted coffee beans

As mentioned in the methods (Chapter 2, 2.2.4), L value ranges from 0 to 100, which represents the relevant colour is from black (0) to white (100). As a result, the colour turns darker with the decreasing of L value. Table 5.2 showed the effects of fertilizer levels and shade tree types on the colour of roasted coffee beans.

With regards to the different fertilizer levels, L values of roasted coffee beans were significantly lower ($P<0.05$) under the intensive conventional (IC) (51) and the intensive organic (IO) (50) management than under the moderate conventional (MC) (54) management. As a result, the colour of roasted coffee beans were significantly darker ($P<0.05$) under the intensive conventional (IC) and the intensive organic (IO) management than under the moderate conventional (MC) management. Nevertheless, no significant differences ($P<0.05$) were detected in the L values between the intensive organic (IO) and low organic (LO), and the intensive conventional (IC) and intensive organic (IO).

In terms of shade types, the roasted coffee beans under the full sun system (53) had a significantly lower ($P<0.05$) L value than under the shaded (57) and *Erythrina* (58) systems. Thus, the colour of roasted coffee beans was significantly darker ($P<0.05$) under the full sun system than under the shaded and *Erythrina* systems. Furthermore, the L value of roasted coffee beans was significantly lower ($P<0.05$) under the service shade tree system (51) than under the timber shade tree system (57), while there was a significantly higher

(P<0.05) L value detected in the roasted coffee beans under the non-legume timber shade tree system (59) than under the legume timber shade tree system (52). Therefore, the colour of roasted coffee beans was significantly darker (P<0.05) under the service shade tree system than under the timber shade tree system, whilst the roasted coffee beans under the non-legume timber shade tree system had a significantly lighter (P<0.05) colour than under the legume shade tree system.

Table 5.2 Contrast results for the colour of roasted coffee beans; Values were presented as mean, standard error of the contrast difference ($S.E._D$) and significance of the difference (P-value); P-values <0.05 were shown in bold.

Contrast Managements	L value Mean	$S.E._D$	p-value
IC vs MC	51 54	2	**0.042**
MC vs IO	54 50	2	**0.006**
IO vs LO	53 54	1	0.085
IC vs IO	53 54	2	0.102
Shade types			
FS vs S	53 57	3	**0.016**
E vs FS	58 53	1	**<.001**
Ser. vs Tim.	51 57	3	**<.001**
LT vs NLT	52 59	1	**<.001**

IC: Intensive Conventional, MC: Moderate Conventional, IO: Intensive Organic, LO: Low Organic; FS: Full Sun, S: Shaded, E: *Erythrina poepiggiana*, Ser.: Service, Tim: Timber, LT: Legume Timber, NLT: Non-Legume Timber.

5.3 Discussions

Although many complex chemical reactions such as the degradation of sugar and acids occur during coffee bean roasting progress, Maillard reaction is obviously one of the most important chemical reactions to promote the formation of coffee flavour and colour (Dart and Nursten, 1985; Grosch, 2001). Maillard reaction is related to the interactions between reducing sugars and amino acids (Maillard, 1912). Even though there is a large amount of polysaccharides present in the green coffee beans (Bradbury and Halliday, 1987), most carbohydrates are relatively stable to roasting (Bradbury and Halliday, 1990; Wilson et al., 1997) and therefore it is the relative abundance of free or available sugars drives the thermal flavour generation. Moreover, on roasting, sucrose is rapidly degraded and its content dramatically decreases by about 97% (Trugo and Macrae, 1985). As a result, during coffee bean roasting, the reducing sugars and amino acids, as the main precursors of Maillard reaction, are mainly derived from the decomposition of sucrose and protein in the green coffee beans, respectively. Furthermore, the melanoidins, which contributes to the colour formation of roasted coffee beans, are derived from Maillard reaction and dehydration of sucrose (Bradbury, 2001). Therefore, it is proposed that the contents of sucrose and total protein in the green coffee beans are responsible for the formation of coffee flavour and colour.

5.3.1 How fertilizer levels and shade types affect the formation of volatile compounds and colour of roasted coffee beans

There were no significant differences ($P<0.05$) detected in the sucrose content of green coffee beans among the different fertilizer levels and shade types (see Table 4.6 in Chapter 4). Nonetheless, significant differences ($P<0.05$) were found in the total protein content of green coffee beans among the different fertilizer levels and shade types (see Table 4.7 in Chapter 4).

In terms of the different fertilizer levels, the total protein content of green coffee beans was significantly lower ($P<0.05$) under the moderate conventional (MC) management than under the intensive conventional (IC) and intensive organic (IO) management. This explains why the roasted coffee beans under moderate conventional (MC) management presented a significantly lower ($P<0.05$) amount of volatile compounds and lighter colour than those roasted coffee beans under the intensive conventional (IC) and intensive organic (IO) management.

With regards to the different shade types, the amounts of volatile compounds in the roasted coffee beans were significantly higher ($P<0.05$) and the colour of roasted coffee beans was significantly darker ($P<0.05$) under the full sun system than under the shaded and *Erythrina* system due to a significantly higher ($P<0.05$) total protein content of green coffee beans in the full sun system. Similarly, due to the significantly higher ($P<0.05$) total protein content of green coffee beans under the service and legume timber shade tree system, the roasted coffee beans under the service and legume timber shade tree system had a significantly higher ($P<0.05$) amount of volatile compounds and

significantly darker ($P<0.05$) colour than those roasted coffee beans under the timber and non-legume timber shade tree system, respectively.

To sum up, in our experiment, the total protein content of green coffee beans was significantly affected by the fertilizer levels and shade types, while there was no significant effect on the sucrose content of green coffee beans. As a result, the total protein content was the main factor to drive the formation of aroma and colour of roasted coffee beans. As has been discussed in the Chapter 4 (4.3.5), the green coffee beans under the intensive conventional (IC) and intensive organic (IO) management had a significantly higher ($P<0.05$) total protein content than those under the moderate conventional (MC) management due to the higher fertilizer input amount, especially the nitrogen. Thus, the higher N input amount could increase the protein generation in the green coffee beans and further improve the formation of final flavour and colour of roasted coffee beans. Furthermore, the legume shade tree could improve the protein generation in the green coffee beans by fixing more N_2 and further promote the formation of final flavour and colour of roasted coffee beans. Compared with shaded system, the light intensity of full sun system was significant higher, which improved the protein biosynthesis in the ribosome, and finally, promoted the formation of flavour and colour of roasted coffee beans.

5.4 Conclusions

Sixty-four volatile aroma compounds were detected in the roasted coffee beans. The roasted coffee beans from the intensive conventional (IC) and intensive organic (IO) management had a significantly higher ($P<0.05$) amount of volatile compounds and significantly darker ($P<0.05$) colour than those roasted coffee beans from the moderate conventional (MC) management due to the significantly higher ($P<0.05$) total protein content of green coffee beans under the intensive conventional (IC) and intensive organic (IO) management. Similarly, due to the significantly higher ($P<0.05$) total protein content of green coffee beans under the full sun, service shade tree and legume shade tree system, the roasted coffee beans from these shade types had a significantly higher ($P<0.05$) amount of volatile compounds and significantly darker ($P<0.05$) colour than those roasted coffee beans from the shaded, *Erythrina*, timber shade tree and non-legume shade tree system.

In this chapter, the relationship between the different fertilizer levels and shade types of coffee plants and volatile compounds generation during roasting of green coffee beans has been explored. Next chapter (Chapter 6) discusses the effects of different fertilizer levels and shade types on the coffee yield and morphology, the physical and chemical attributes of green coffee beans and further volatile compounds generation of roasted coffee beans.

Chapter 6 Discussions

6.1 Effects of fertilizer levels on coffee plant morphology, yield, physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans. To what extent can organic management compete with conventional management?

A general view in coffee agriculture is that organic management produces a lower yield than its conventional counterpart (Blackman and Naranjo, 2012). In our experiment, although the input amounts of phosphorus (P) and potassium (K) were significantly higher and only slightly lower nitrogen (N) input amounts in the intensive organic (IO) management, the intensive organic (IO) yield was always inferior to the intensive conventional (IC) management. Nonetheless, after several years later (about 4-5 years), the intensive organic (IO) management showed a similar and even higher yield to the moderate conventional (MC) management which received half the amount of the intensive conventional (IC) fertilizer input. The coffee plant morphology was worse under the intensive organic (IO) management for the first 2 years than under the intensive conventional (IC) and moderate conventional (MC) managements. However, after a long period, in 2014, the performance of the coffee plant morphology improved and was similar to the moderate conventional (MC) managements. Additionally, the BI yield, which represented the production stability, always remained lower under the intensive organic (IO) management than under the intensive conventional (IC) and moderate conventional (MC) managements.

It is commonly reported by the coffee farms in Costa Rica that the slower release of plant available nitrogen from organic inputs (Seufert et al., 2012), better availability of chemical fertilizers and the positive correlation of coffee production and nitrogen fertilizer input may be the reasons to explain the better performance of the intensive conventional (IC) management compared to the moderate conventional (MC) and organic managements (Castro-Tanzi et al., 2012). However, a similar experiment was undertaken by Haggar et al. (2011) in Nicaragua where the organic and non-organic coffee received the same total amounts of nitrogen input, and they found a similar coffee yields between the intensive conventional (IC) and organic coffee managements. The differences between these results may because the Nicaragua site was previously used for coffee production and had higher organic matter and better soil conditions. In addition, Lyngbaek et al. (2001) stated that even though organic coffee produced lower coffee yields, some organic management showed similar or even higher production than their inorganic counterparts.

In our experiment, the moderate conventional (MC) management only had the better performance than the intensive organic (IO) management in the early stage of production (the first two years of production) due to the already mentioned longer release period of organic fertilizers and the time to recover organic matter in soil after the previous management of sugar cane plantation (Haggar et al, 2011). Moreover, it is a widely reported phenomenon of the lower initial yields and incremental productivity over time in organic management (Seufert et al., 2012).

In terms of the physical and chemical attributes of green coffee beans, organic

and non-organic managements had no significant effect on the physical attributes (density, bulk density, moisture content and roasting loss) and carbohydrates contents of green coffee beans. The phosphorus (P), potassium (K) and calcium (Ca) contents in the green coffee beans were significantly higher under the intensive organic (IO) management than under the intensive conventional (IC) and moderate conventional (MC) managements due to a higher fertilizer input amount. It was similar to the result of soil analysis from Haggar et al. (2011) that the higher amounts of phosphorus (P), potassium (K) and calcium (Ca) were detected in the soil under the intensive organic (IO) management. In addition, Haggar et al. (2011) also stated that chicken manure had up to 10% calcium, which resulted in the relatively high calcium (Ca) content in soil and green coffee beans under the intensive organic (IO) management. The contents of manganese (Mn), copper (Cu) and zinc (Zn) in the green coffee beans were significantly lower under the intensive organic (IO) management than under the intensive conventional (IC) and moderate conventional (MC) managements, while the molybdenum (Mo) content in the green coffee beans was significantly higher under the intensive organic (IO) management, as the organic management improves the pH of soil. The unpublished data of soil acidity analysis from Haggar in Costa Rica confirmed the organic management decreased the acidity of soil and increased the pH.

Although the total lipid and fatty acids contents in the green coffee beans were not significantly different between the intensive organic (IO) and intensive conventional (IC) management. When, comparing to the moderate conventional (MC) management, the green coffee beans under the intensive organic (IO) management had a significantly higher total lipid and elevated

level of palmitic acid and oleic acid due to the higher fertilizer levels. Furthermore, similar results were observed in the total protein and caffeine contents due to the higher N-fertilizer inputs in the intensive organic (IO) management. Müller and Hippe (1987) stated that the protein content increased with increasing nitrogen application. In addition, Nitika et al. (2008) reported that the higher protein content was found in the organic wheat. The amounts of volatile aroma compounds in roasted coffee beans, therefore, were significant higher and the colour of roasted coffee beans was significantly darker under the intensive organic (IO) management than the moderate conventional (MC) management due to high protein improving the Maillard reaction, whilst no significant differences were detected between the intensive organic (IO) and the intensive conventional (IC) management.

In conclude, comparing with the intensive conventional (IC) management, the intensive organic (IO) management had worse productivity (yield and morphology), but showed a more stable production. A similar result could be observed in the study of Haggar et al. (2011) who measured coffee yield in the early stage of coffee production in Costa Rica. However, with regards to some physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans, there were no significant differences between these two managements. After a long period management, the intensive organic (IO) management showed a similar productivity to the moderate conventional (MC) management and better production stability. Moreover, in terms of the contents of several chemical nutrients such as the total lipid and protein in the green coffee beans and the ability to generate more pleasant volatile compounds and colour, the intensive organic (IO)

management had a better performance than the moderate conventional (MC) management. As a result, to some extent, the intensive organic (IO) management could be used to replace completely the moderate conventional (MC) management. In addition, when the very high cost of intensive conventional (IC) management was considered as a limitation factor to coffee farmers, the intensive organic (IO) management might be an alternation.

6.2 Comparing full sun and shaded system for coffee plant morphology, yield, physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans

Comparing with the shaded system, the average coffee yield in 13 years was increased by 23-37% in the full sun system due to the higher light availability and no competition between coffee plants and shade trees. Nevertheless, the shaded system showed stronger production stability. For coffee plant morphology, in 2002, the coffee plants under the full sun system had a slightly better performance, while, in the later stage (2014), no significant differences were detected between the full sun and shade system.

Under the optimal site conditions, compared with the full sun system, the shaded system produces lower yields due to the lower light intensity and competition between the shade trees and coffee plants (Vaast et al., 2005; DaMatta, 2004). However, under the sub-optimal site conditions, shade is regarded as the essential system for a sustained coffee production due to it improving adverse site conditions (reduction of variation of temperature between day and night; protection from wind; reduction in growth of weeds) (de Carvalho Gomes et al., 2016; Lin, 2007), which results in a similar or even higher production under shade (DaMatta, 2004; Vaast et al., 2005). Optimal growing conditions for Arabica coffee mean that the annual rainfall and temperature are in the range of 1200-1800 mm and 18-21 °C, separately (Alègre, 1959). Turrialba in Costa Rica with 2915 mm/year rainfall and mean annual temperature of 22 °C can thus be considered as a suboptimal site due to

a significant higher mean annual rainfall and a slightly higher average temperature. However, even though the Costa Rica field was a suboptimal site, possible positive impacts of shade system were not observed in our study due to the lower light availability, if conventional management practices were applied.

Beer et al., (1997) and Campanha et al. (2004) stated that the lower light availability in shaded systems restricted the development of nodal and flower bud and consequently coffee yield, while Morais et al. (2003) and Vaast et al. (2005) reported that shaded systems improved the vegetative development of coffee plant (height, number of branches/leaves and biomass). Other cited possibilities for lower production under the shaded systems such as the competition of water and nutrients between the shade trees and coffee plants may not be the reasons to explain the low yields under the shaded systems in our experiment due to the abundant rainfall and high input level of conventional management (IC, MC).

In terms of the physical attributes of green coffee beans, the density of green coffee beans was significantly higher under the full sun system due to the lower moisture content of green coffee beans driven by the higher temperature and light intensity. The high temperature and light intensity can increase leaf-to-air vapour pressure and improve the stomatal opening of coffee leaves and consequently result in the rising of transpiration rates. Thus, water loss caused by exceeding coffee tree evapotranspiration will increase (Maestri et al, 2001). The density of green coffee bean is around 1300-1400 Kg/m^3 (Sivetz and Desrosier, 1979; Clarke and Macrae, 1988; Dutra et al., 2001) which is higher

than the water density (1000 Kg/m^3). As a result, the green coffee bean density would increase with the decreasing moisture content. The higher density of green coffee beans is beneficial to increase the coffee farmers' income in the international trade, as the green coffee beans are evaluated by weight instead of volume.

With regards to the chemical attributes of green coffee beans, the higher temperature and light intensity in the full sun system drove the absorbance of some minerals such as sulfur (S), calcium (Ca) and manganese (Mn). There were no significant differences detected in the contents of total lipid, fatty acids and carbohydrates in the green coffee beans between the full sun and shaded system, while the contents of total protein and caffeine in the green coffee beans were significantly higher under the full sun system than under the shaded system. As a result, the roasted coffee beans under the full sun system generated more volatile aroma compounds and a darker colour.

However, Vaast et al. (2006) presented that caffeine and total lipid contents were significantly higher in green coffee beans under the shaded system, while sucrose content was significantly higher in green coffee beans under the full sun system. In addition, a similar negative relationship between total lipid and sucrose has been reported by Montagnon et al. (1998) and Bertrand (2002). It was explained by these authors that a high sucrose content coupled with a low total lipid content in green coffee beans under the full sun system indicated bean filling and lipid biosynthesis were not fully achieved, as sucrose was regarded as a precursor of polymer sugar and lipid compounds in green coffee beans and shaded system improved the maturation of coffee cherries by

prolonging ripeness period. The differences between previous researches and this study may due to the fact that only conventional managements (IC, MC) were combined with the full sun system and high input of chemical fertilizers could improve the ripeness of coffee cherries.

To sum up, the full sun system is regards as the first choice for improving coffee farmers' income by increasing the coffee yield as well as the density of green coffee beans. In addition, the roasted coffee beans under the full sun system showed the more volatile aroma compounds and a darker colour. However, in the full sun system, the increase of productivity is achieved by using a large number of external agrochemical inputs and shortcutting ecological cycles (Beer et al., 1997; Haggar et al., 2011) which contributes to environmental pollution, degradation of soils (Lyngbaek et al., 2001; DaMatta, 2004) and health hazards (Nygern, 2012). The high annual cost caused by these high external inputs cannot be reduced easily, which results in vulnerability of coffee farmers to the unstable international coffee market (Haggar et al., 2011). Shaded system, therefore, is considered as the essential option for a sustainable coffee production, as it improves adverse site conditions such as temperature, humidity and soil conditions. Furthermore, shaded system also maintains the production stability due to the smaller fluctuation and lower bienniality index (BI) of coffee yield. Haggar et al. (2011) stated in the early stage the similar smaller fluctuation and lower bienniality index (BI) of coffee yield was observed under the shaded systems in Costa Rica site.

6.3 Comparing full sun and *Erythrina* system for coffee plant morphology, yield, physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans

The *Erythrina* system was selected to compare with the full sun system individually due to the very low shade cover (18.4%). Although the production of *Erythrina* system was more stable due to the lower BI yield, the mean 13 coffee yield was significantly higher under the full sun system than under the *Erythrina* system. Nevertheless, for the morphology of coffee plant, both in 2002 and 2014, no significant differences were detected between these two systems.

The small differences of shade cover between the *Erythrina* and full sun systems still led to the significantly higher coffee yields under the full sun system, which meant that light intensity was the most important factor to drive the coffee production (Vaast et al., 2005; DaMatta, 2004). Beer et al., (1988) and Campanha et al. (2004) stated that the lower light availability in shaded systems restricted the development of nodal and flower bud and consequently coffee yield, while Morais et al. (2003) and Vaast et al. (2005) reported that shaded systems improved the vegetative development of coffee plant (height, number of branches/leaves and biomass).

In terms of the physical and chemical attributes of green coffee beans, the green bean density was still significantly higher under the full sun system than under the *Erythrina* system due to the lower moisture content present in the full sun system. The high temperature and light intensity can increase leaf-to-air

vapour pressure and improve the stomatal opening of coffee leaves and consequently result in the rising of transpiration rates. Thus, water loss caused by exceeding coffee tree evapotranspiration will increase (Maestri et al, 2001). The density of green coffee bean is around 1300-1400 Kg/m^3 (Sivetz and Desrosier, 1979; Clarke and Macrae, 1988; Dutra et al., 2001) which is higher than the water density (1000 Kg/m^3). As a result, the green coffee bean density would increase with the decreasing moisture content.

The contents of sulfur (S), calcium (Ca) and manganese (Mn) were significantly higher under the full sun system than under the *Erythrina* system due to the relatively higher temperature and light intensity, while the contents of copper (Cu), zinc (Zn) and sodium (Na) were significantly lower under the full sun system than under the *Erythrina* system due to the calcium (Ca) restriction. The ability of plant to absorb minerals increases with the increasing temperature when temperature is below 40°C (Starck et al., 1994). Moreover, the higher light intensity improves the absorptivity of minerals in plant by promoting transpiration (Caruso et al., 2003). Lynch and Läuchli (1984) stated that more calcium (Ca) intake in plant restricted the absorption of copper (Cu), zinc (Zn) and sodium (Na). In addition, there were no significant differences detected in the total lipid content of green coffee beans between the full sun system and the *Erythrina* system, whilst the fatty acids contents were significantly higher under the *Erythrina* system than under the full sun system due to the replenishment of organic matters to the site by the intensive pruning of *Erythrina* shade tree. Similar results were also found in the polysaccharides contents. Haggar et al. (2011) stated that the strong pruning of shade tree could improve the nutrient recycling and compensate the organic matter to the site.

Nonetheless, the total protein content of green coffee beans was significantly higher under the full sun system than under the *Erythrina* system due to the higher light intensity improving the protein biosynthesis in the ribosome (Boulter. 1970), although the *Erythrina* system could supply some organic matters to the soil. As a result, the roasted coffee beans under the full sun system contained the more volatile aroma compounds and a darker colour.

To sum up, comparing with the full sun system, although the shade cover of *Erythrina* system was very low, the *Erythrina* system still had the significantly lower coffee yield and green bean density. The only advantage of *Erythrina* system was to replenish the organic matters to improve the fatty acids and polysaccharides contents of green coffee beans through the intensive pruning. Nonetheless, due to the leading role of light intensity, the total protein content was significant higher under the full sun system than under the *Erythrina* system. Thus, the roasted coffee beans under the *Erythrina* system showed the less volatile aroma compounds and a lighter colour.

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6.4 Comparing service and timber system for coffee plant morphology, yield, physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans

The average 13 years coffee yield was significantly higher under the service system than under the timber system due to the higher light intensity, the replenishment of organic matters by intensive pruning of service trees and the lower competition between coffee plants and shade trees, while no significant difference was found in BI yield between these two shade systems. In terms of the morphology of coffee plant, in 2002, there were no significant differences between the service and timber system, while, in the later stage (2014), the coffee plant had a better performance under the service system than under the timber system.

When comparing service and timber system, coffee performance had a clear shift between the initial and late development stage of the plantation (Fig 3.2 (c)). As a result, long-term observations are essential for evaluating the performance of agro-forestry systems. The similar or even higher coffee yields were observed under the timber tree system than service tree system in the first 6 years. Haggar et al. (2011) who managed the early stage of the experiment, drew the conclusion that timber tree system might be a better option due to the revenue of timber sales and higher competition between service trees and coffee plants (higher shade cover prior to pruning and higher basal area than timber trees). However, in the later stage (2008 onwards), this pattern totally shifted to the higher production and better performance of coffee morphology

under the service system.

In our experiment, service shade tree (*Erythrina*) was pruned heavily and partially every year, while only the lower branches of shade trees were pruned in timber shade tree system. In addition, the expanding growth of the timber tree crowns increased the competition between timber shade trees and coffee plants, while the competition remained in the service shade tree system. After a long period, on the one hand, significantly lower shade cover was present in the service shade tree system; on the other hand, a stronger competition between shade trees and coffee plants appeared in the timber shade tree system. As a result, the higher coffee yield and better coffee morphology was observed in the service shade tree system. Vaast et al. (2005) also reported that after an investigation of 100 farms in Costa Rica, there were not acceptable coffee yields under the timber shade tree system due to higher shade cover. Nevertheless, other factors such as higher biomass inputs and nutrient recycling by strong pruning and litter fall in the service system might be some reasons to improve the higher yields and better coffee morphology on the long term.

In terms of the physical and chemical attributes, the moisture content of green coffee beans under the service system was significantly lower than under the timber system due to the higher light intensity. The high temperature and light intensity can increase leaf-to-air vapour pressure and improve the stomatal opening of coffee leaves and consequently result in the rising of transpiration rates. Thus, water loss caused by exceeding coffee tree evapotranspiration will increase (Maestri et al, 2001). The density of green coffee bean is around 1300-

1400 Kg/m^3 (Sivetz and Desrosier, 1979; Clarke and Macrae, 1988; Dutra et al., 2001) which is higher than the water density (1000 Kg/m^3). As a result, the green coffee bean density would increase with the decreasing moisture content. Nevertheless, the total lipid and fatty acids contents and the total protein contents in the green coffee beans were significantly higher under the service system than under the timber system due to the higher light intensity and the supplement of organic matters by strong pruning of service shade tree. As a result, the roasted coffee beans under the service system had more volatile aroma compounds and a darker colour.

In conclusion, comparing with the timber shade tree, the greatest advantage of service shade tree was to replenish the organic matters to the site by the intensive pruning. As a result, the total protein content of green coffee beans under the service system was significantly higher, which resulted in the formation of more volatile aroma compounds and a darker colour in the roasted coffee beans. In addition, the higher light intensity caused by the lower shade cover was another factor to improve the coffee yield and morphology performance of coffee plant.

6.5 Comparing legume and non-legume system for coffee plant morphology, yield, physical and chemical attributes of green coffee beans and the formation of flavour and colour of roasted coffee beans

The coffee yield was not significantly different between the intensive organic (IO) and low organic (LO) managements when combined with the legume species *Erythrina* and *Chloroleucon*, while the low organic (LO) management collapsed totally under the non-legume species *Terminalia*. Under the low organic (LO) input conditions, the legume shade trees may replenish the low external input through N_2 fixation (Nygren et al., 2012). Tully and Lawrence (2012) stated that the *Erythrina* shade tree could compensate 70 to 90 Kg N ha^{-1} $year^{-1}$. Additionally, when *Erythrine* tree was used, strong pruning and nutrient recycling could improve the external nutrient input. However, when the low organic (LO) management compared with the non-legume species *Terminalia*, N-availability was most likely too low to maintain coffee growth over time, which resulted in the collapse of coffee plants. As a result, legume shade tree has positive impacts on the coffee production in low external input systems.

In terms of the physical and chemical attributes of green coffee beans, the legume shade tree increased the total protein content of green coffee beans by N_2 fixation and resulted in the increasing of volatile aroma compounds and a darker colour in the roasted coffee beans. As a result, the green coffee beans under the legume shade tree system had significant higher total protein content than under the non-legume shade tree system, and correspondingly, the roasted

coffee beans under the legume shade tree system had more volatile aroma compounds and a darker colour.

Chapter 7 Conclusions and future work

7.1 Conclusions

Organic management always showed a more stable production, which would promote stability of the coffee farmers' income. Coffee yield and morphology were always worse under the intensive organic (IO) management than under the intensive conventional (IC) management. Nevertheless, after a long management period, the intensive organic (IO) management did not only have a similar or even higher productivity to the moderate conventional (MC) management, but also had more lipids, proteins and caffeine in the green coffee beans and a stronger ability to generate more volatile aroma compounds and a darker colour. Therefore, the intensive organic (IO) management can be considered as an excellent substitution of the moderate conventional (MC) management. Additionally, to some extent, the intensive organic (IO) management might be a good option, when there was a cost limitation of the intensive conventional (IC) management.

The full sun system was the first option for improving coffee farmers' income due to the high coffee yield and density of green coffee beans as well as the more volatile aroma compounds and a darker colour, although the production stability was relatively low. Nevertheless, the shaded system might be a crucial choice for maintaining a sustainable coffee production by ameliorating the adverse site conditions.

The greatest advantage of the service or *Erythrina* system (the *Erythrina* was the only service tree in our experiment) was the replenishment of organic

matter to the soil by extremely strong pruning. In spite of this, the coffee yield and green bean density were still significantly lower under the service or *Erythrina* system than under the full sun system. Furthermore, the roasted coffee beans under the full sun system had more volatile aroma compounds and a darker colour. The supplement of organic matters only improved the fatty acids and polysaccharides contents of green coffee beans, when comparing with the full sun system. Nonetheless, when comparing with the timber system, the service or *Erythrina* system did not only have the higher coffee yield and better morphology of the coffee plant, but it also produced the green coffee beans with the higher total protein content. As a result, the roasted coffee beans under the service or *Erythrina* system had more volatile aroma compounds and a darker colour than under the timber system.

Comparing with the non-legume shade tree, legume shade tree directly influenced the total protein content of green coffee beans by fixing N_2 and improved the performance of flavour and colour of roasted coffee beans.

7.2 Future work

7.2.1 Impact of coffee plant pruning on the physical and chemical attributes of green coffee beans and flavour and colour generation of roasted coffee beans

In our experiment, the samples from both 2013 and 2014 were analysed. In 2013, the coffee plants received the relatively strong pruning, while, in 2014, the pruning intensity was weaker. When the two years data were analysed separately, there were some differences between the years. However, we only had one year data for each strong and weak pruning of coffee plants. They was not enough data to find the possible effects of pruning intensity of coffee plants on the physical and chemical attributes of green coffee beans and flavour and colour generation of roasted coffee beans. Therefore, in the future, more samples under both strong and weak pruning conditions should be collected from the field and analysed.

7.2.2 More minerals should be involved

In our experiment, twenty-eight minerals were found, but only eleven minerals were analysed. Nevertheless, the other minerals can play an essential role in human health. During the coffee bean roasting progress, the minerals are relatively stable and their contents do not decrease. In addition, minerals can be dissolved into the water. Therefore, coffee beverage can be considered as a drink to supply human for essential mineral elements. As a result, it is quite meaningful to explore the effect of fertilizer levels and shade types on the various minerals in the green coffee beans.

7.2.3 Lipidomics analysis

In this thesis, only the total lipid and fatty acids were analysed and reported. However, the lipidomics analysis of green coffee beans from the different agro-forestry systems has been done. The data has not been showed in this thesis due to the deficiency of time. Around 7200 possible lipids were found in our experiment, but have not been identified. The lipids play a crucial role in aroma generation of roasted coffee beans, as the autoxidation of lipids during storage and transportation of green coffee beans results in the formation of volatile aldehydes and other compounds, which may be involved in further reactions. Moreover, during the coffee bean roasting, lipids can protect the volatile aroma compounds. Therefore, it is necessary to investigate the lipidomics in our green coffee bean samples.

7.2.4 Amino acids analysis

In our experiment, the total protein content was significantly different among the different fertilizer levels and shade types. In addition, in chapter 5, the total protein content has been suggested the main factor to drive the flavour and colour generation of roasted coffee beans. Therefore, it is essential to analyse the amino acids composition in our green coffee bean samples. These experiments have not been done, as the cost of amino acids analysis is a limited factor and we had a huge sample capacity (117 bags of samples including the replicates in the field). Furthermore, due to the huge sample pool, the analysis of amino acids, carbohydrates and aroma generation in our samples may improve the further understanding of Maillard reaction mechanisms especially in the later reaction stage, which has not been investigated clearly.

7.2.5 Analysis of coffee acids

The acids such as chlorogenic acids and quinic acid in the green coffee beans also play an essential role in aroma generation of roasted coffee beans, and they will affect the quality of final product. Thus, it is necessary to study the effect of fertilizer levels and shade types on the acids composition of green coffee beans.

Appendix I The quantitative data for volatile aroma compounds in different contrast models

Table 1 The quantitative data for volatile aroma compounds in the contrast group IC vs MC

	IC (mg/kg)	MC (mg/kg)
2-Methylfuran	0.0185	0.0184
p-Cresol	0.0069	0.0069
Diacetyl	0.0529	0.0488
2,3-Pentanedione	0.0927	0.0918
Dimethyl Disulphide	0.0027	0.0026
2-Vinylfuran	0.0054	0.0053
Vinylpyrazine	0.0021	0.0021
2,3-Hexanedione	0.0049	0.0047
1-Methylpyrrole	0.0095	0.0093
2,5-Dimethylfuran	0.0024	0.0023
2-Ethyl-3,6-dimethylpyrazine	0.0030	0.0024
2,4,5-Trimethyloxazole	0.0008	0.0008
2-Pentylfuran	0.0003	0.0002
2-Methoxymethylfuran	0.0015	0.0015
2-Methylpyrazine	0.3159	0.3121
Dihydro-2-methyl-3-furanone	0.0520	0.0505
4-Methylthiazole	0.0025	0.0023
2,6-Diethylpyrazine	0.0005	0.0004
2,5-Dimethylpyrazine	0.0361	0.0316
2,6-Dimethylpyrazine	0.0710	0.0684
2-Ethylpyrazine	0.0383	0.0372
2,3-Dimethylpyrazine	0.0128	0.0121
2-Methyl-2-cyclopentenone	0.0015	0.0014
2-Ethyl-6-methylpyrazine	0.0136	0.0121
2-Ethyl-5-methylpyrazine	0.0089	0.0080
2,3,5-Trimethylpyrazine	0.0101	0.0090
2-Ethyl-3-methylpyrazine	0.0080	0.0071
Propylpyrazine	0.0298	0.0260
Acetoin	0.0259	0.0238
Hexanal	0.0007	0.0005
4-Ethylguaiacol	0.0001	0.0001
Pyrrole	0.0090	0.0080
Acetic acid	0.3961	0.3471

Furfural	0.4162	0.3492
Acetoxyacetone	0.1122	0.1102
2-Fufurylmethyl sulfide	0.0009	0.0008
2-Acetylfuran	0.0347	0.0296
2-Ethyl-3,5-dimethylpyrazine	0.0007	0.0006
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0004
Acetoxy-2-butanone	0.0160	0.0158
2-Furfurylacetate	0.0235	0.0231
Propionic acid	0.0105	0.0089
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0792	0.0777
2-Acetylpyridine	0.0005	0.0005
1-Methyl-2-formylpyrrole	0.0031	0.0030
g-Butyrolactone	0.0093	0.0090
Furfuryl alcohol	0.2179	0.1891
Isovaleric acid	0.0287	0.0232
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0057	0.0052
1-Furfurylpyrrole	0.0011	0.0011
2-Methoxy-4-vinylguaiacol	0.0009	0.0009
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0018	0.0017
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0021	0.0021
Pyridine	0.0675	0.0653
Guaiacol	0.0004	0.0004

Table 2 The quantitative data for volatile aroma compounds in the contrast group MC vs IO

	MC (mg/kg)	IO (mg/kg)
2-Methylfuran	0.0192	0.0197
p-Cresol	0.0079	0.0083
Diacetyl	0.0506	0.0559
2,3-Pentanedione	0.0871	0.0960
Dimethyl Disulphide	0.0030	0.0030
2-Vinylfuran	0.0058	0.0059
Vinylpyrazine	0.0023	0.0022
2,3-Hexanedione	0.0050	0.0051

1-Methylpyrrole	0.0099	0.0092
2,5-Dimethylfuran	0.0025	0.0026
2-Ethyl-3,6-dimethylpyrazine	0.0025	0.0032
2,4,5-Trimethyloxazole	0.0009	0.0009
2-Pentylfuran	0.0003	0.0003
2-Methoxymethylfuran	0.0016	0.0017
2-Methylpyrazine	0.3174	0.3222
Dihydro-2-methyl-3-furanone	0.0491	0.0540
4-Methylthiazole	0.0024	0.0025
2,6-Diethylpyrazine	0.0004	0.0005
2,5-Dimethylpyrazine	0.0318	0.0355
2,6-Dimethylpyrazine	0.0669	0.0715
2-Ethylpyrazine	0.0367	0.0399
2,3-Dimethylpyrazine	0.0122	0.0132
2-Methyl-2-cyclopentenone	0.0015	0.0016
2-Ethyl-6-methylpyrazine	0.0127	0.0146
2-Ethyl-5-methylpyrazine	0.0082	0.0095
2,3,5-Trimethylpyrazine	0.0092	0.0106
2-Ethyl-3-methylpyrazine	0.0073	0.0085
Propylpyrazine	0.0265	0.0299
Acetoin	0.0251	0.0274
Hexanal	0.0005	0.0006
4-Ethylguaiacol	0.0001	0.0001
Pyrrole	0.0082	0.0091
Acetic acid	0.3654	0.4246
Furfural	0.3743	0.4458
Acetoxyacetone	0.1185	0.1239
2-Fufurylmethyl sulfide	0.0009	0.0009
2-Acetylfuran	0.0323	0.0380
2-Ethyl-3,5-dimethylpyrazine	0.0006	0.0007
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0005
Acetoxy-2-butanone	0.0172	0.0179
2-Furfurylacetate	0.0250	0.0256
Propionic acid	0.0098	0.0118
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0785	0.0885
2-Acetylpyridine	0.0006	0.0006
1-Methyl-2-formylpyrrole	0.0033	0.0034
g-Butyrolactone	0.0098	0.0098
Furfuryl alcohol	0.2126	0.2158
Isovaleric acid	0.0208	0.0271
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0054	0.0060

1-Furfurylpyrrole	0.0012	0.0012
2-Methoxy-4-vinylguaiacol	0.0010	0.0010
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0020	0.0019
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0023	0.0023
Pyridine	0.0690	0.0701
Guaiacol	0.0005	0.0005

Table 3 The quantitative data for volatile aroma compounds in the contrast group IO vs LO

	IO (mg/kg)	LO (mg/kg)
2-Methylfuran	0.0200	0.0197
p-Cresol	0.0075	0.0073
Diacetyl	0.0517	0.0505
2,3-Pentanedione	0.0913	0.0890
Dimethyl Disulphide	0.0029	0.0028
2-Vinylfuran	0.0056	0.0055
Vinylpyrazine	0.0022	0.0021
2,3-Hexanedione	0.0050	0.0049
1-Methylpyrrole	0.0103	0.0090
2,5-Dimethylfuran	0.0025	0.0025
2-Ethyl-3,6-dimethylpyrazine	0.0031	0.0031
2,4,5-Trimethyloxazole	0.0009	0.0009
2-Pentylfuran	0.0003	0.0003
2-Methoxymethylfuran	0.0015	0.0014
2-Methylpyrazine	0.3230	0.3231
Dihydro-2-methyl-3-furanone	0.0504	0.0498
4-Methylthiazole	0.0026	0.0024
2,6-Diethylpyrazine	0.0005	0.0005
2,5-Dimethylpyrazine	0.0345	0.0347
2,6-Dimethylpyrazine	0.0715	0.0710
2-Ethylpyrazine	0.0392	0.0388
2,3-Dimethylpyrazine	0.0131	0.0128
2-Methyl-2-cyclopentenone	0.0014	0.0014
2-Ethyl-6-methylpyrazine	0.0140	0.0140
2-Ethyl-5-methylpyrazine	0.0091	0.0091
2,3,5-Trimethylpyrazine	0.0104	0.0103

2-Ethyl-3-methylpyrazine	0.0083	0.0083
Propylpyrazine	0.0264	0.0260
Acetoin	0.0250	0.0249
Hexanal	0.0007	0.0005
4-Ethylguaiacol	0.0001	0.0001
Pyrrole	0.0094	0.0083
Acetic acid	0.3593	0.3555
Furfural	0.3610	0.3604
Acetoxyacetone	0.1094	0.1028
2-Fufurylmethyl sulfide	0.0008	0.0009
2-Acetylfuran	0.0309	0.0310
2-Ethyl-3,5-dimethylpyrazine	0.0007	0.0007
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0005
Acetoxy-2-butanone	0.0160	0.0152
2-Furfurylacetate	0.0230	0.0227
Propionic acid	0.0094	0.0094
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0746	0.0726
2-Acetylpyridine	0.0005	0.0005
1-Methyl-2-formylpyrrole	0.0032	0.0032
g-Butyrolactone	0.0088	0.0087
Furfuryl alcohol	0.1933	0.1913
Isovaleric acid	0.0229	0.0204
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0053	0.0052
1-Furfurylpyrrole	0.0011	0.0011
2-Methoxy-4-vinylguaiacol	0.0010	0.0010
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0018	0.0018
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0021	0.0021
Pyridine	0.0738	0.0735
Guaiacol	0.0004	0.0004

Table 4 The quantitative data for volatile aroma compounds in the contrast group IC vs IO

	IC (mg/kg)	IO (mg/kg)
2-Methylfuran	0.0195	0.0193

p-Cresol	0.0078	0.0078
Diacetyl	0.0509	0.0492
2,3-Pentanedione	0.0901	0.0833
Dimethyl Disulphide	0.0029	0.0028
2-Vinylfuran	0.0058	0.0058
Vinylpyrazine	0.0023	0.0022
2,3-Hexanedione	0.0051	0.0052
1-Methylpyrrole	0.0098	0.0097
2,5-Dimethylfuran	0.0025	0.0024
2-Ethyl-3,6-dimethylpyrazine	0.0032	0.0031
2,4,5-Trimethyloxazole	0.0009	0.0007
2-Pentylfuran	0.0003	0.0003
2-Methoxymethylfuran	0.0016	0.0015
2-Methylpyrazine	0.3307	0.2668
Dihydro-2-methyl-3-furanone	0.0518	0.0491
4-Methylthiazole	0.0026	0.0022
2,6-Diethylpyrazine	0.0005	0.0005
2,5-Dimethylpyrazine	0.0363	0.0319
2,6-Dimethylpyrazine	0.0639	0.0643
2-Ethylpyrazine	0.0412	0.0405
2,3-Dimethylpyrazine	0.0137	0.0137
2-Methyl-2-cyclopentenone	0.0015	0.0014
2-Ethyl-6-methylpyrazine	0.0148	0.0145
2-Ethyl-5-methylpyrazine	0.0099	0.0104
2,3,5-Trimethylpyrazine	0.0109	0.0101
2-Ethyl-3-methylpyrazine	0.0092	0.0094
Propylpyrazine	0.0255	0.0249
Acetoin	0.0242	0.0237
Hexanal	0.0005	0.0005
4-Ethylguaiacol	0.0001	0.0001
Pyrrole	0.0087	0.0086
Acetic acid	0.3668	0.3584
Furfural	0.3765	0.3663
Acetoxyacetone	0.1130	0.1099
2-Fufurylmethyl sulfide	0.0009	0.0009
2-Acetylfuran	0.0315	0.0291
2-Ethyl-3,5-dimethylpyrazine	0.0007	0.0007
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0005
Acetoxy-2-butanone	0.0168	0.0165
2-Furfurylacetate	0.0246	0.0239
Propionic acid	0.0099	0.0090
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0798	0.0757

2-Acetylpyridine	0.0006	0.0006
1-Methyl-2-formylpyrrole	0.0034	0.0033
g-Butyrolactone	0.0091	0.0090
Furfuryl alcohol	0.2006	0.1939
Isovaleric acid	0.0215	0.0224
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0054	0.0053
1-Furfurylpyrrole	0.0012	0.0010
2-Methoxy-4-vinylguaiacol	0.0010	0.0010
Phenylethyl alcohol	0.0001	0.0001
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0020	0.0020
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0022	0.0022
Pyridine	0.0730	0.0700
Guaiacol	0.0005	0.0005

Table 5 The quantitative data for volatile aroma compounds in the contrast group full sun vs shaded

	Full Sun (mg/kg)	Shaded (mg/kg)
2-Methylfuran	0.0211	0.0147
p-Cresol	0.0083	0.0047
Diacetyl	0.0538	0.0437
2,3-Pentanedione	0.0977	0.0903
Dimethyl Disulphide	0.0031	0.0020
2-Vinylfuran	0.0061	0.0042
Vinylpyrazine	0.0022	0.0018
2,3-Hexanedione	0.0053	0.0032
1-Methylpyrrole	0.0096	0.0076
2,5-Dimethylfuran	0.0027	0.0020
2-Ethyl-3,6-dimethylpyrazine	0.0028	0.0028
2,4,5-Trimethyloxazole	0.0009	0.0009
2-Pentylfuran	0.0003	0.0002
2-Methoxymethylfuran	0.0016	0.0014
2-Methylpyrazine	0.3221	0.3152
Dihydro-2-methyl-3-furanone	0.0552	0.0498
4-Methylthiazole	0.0025	0.0025
2,6-Diethylpyrazine	0.0005	0.0004
2,5-Dimethylpyrazine	0.0339	0.0334

2,6-Dimethylpyrazine	0.0686	0.0678
2-Ethylpyrazine	0.0389	0.0348
2,3-Dimethylpyrazine	0.0132	0.0128
2-Methyl-2-cyclopentenone	0.0016	0.0015
2-Ethyl-6-methylpyrazine	0.0136	0.0137
2-Ethyl-5-methylpyrazine	0.0088	0.0085
2,3,5-Trimethylpyrazine	0.0100	0.0099
2-Ethyl-3-methylpyrazine	0.0079	0.0076
Propylpyrazine	0.0274	0.0266
Acetoin	0.0266	0.0208
Hexanal	0.0008	0.0005
4-Ethylguaiacol	0.0001	0.0000
Pyrrole	0.0087	0.0073
Acetic acid	0.3991	0.3157
Furfural	0.4109	0.3564
Acetoxyacetone	0.1214	0.0934
2-Fufurylmethyl sulfide	0.0009	0.0004
2-Acetylfuran	0.0349	0.0292
2-Ethyl-3,5-dimethylpyrazine	0.0006	0.0004
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0003
Acetoxy-2-butanone	0.0176	0.0115
2-Furfurylacetate	0.0243	0.0164
Propionic acid	0.0110	0.0076
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0811	0.0592
2-Acetylpyridine	0.0006	0.0005
1-Methyl-2-formylpyrrole	0.0032	0.0023
g-Butyrolactone	0.0093	0.0075
Furfuryl alcohol	0.2090	0.1914
Isovaleric acid	0.0266	0.0228
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0057	0.0056
1-Furfurylpyrrole	0.0012	0.0011
2-Methoxy-4-vinylguaiacol	0.0010	0.0010
Phenylethyl alcohol	0.0002	0.0001
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0019	0.0018
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0022	0.0021
Pyridine	0.0706	0.0698
Guaiacol	0.0005	0.0005

Table 6 The quantitative data for volatile aroma compounds in the contrast group *Erythrina* vs full sun

	Erythrina (mg/kg)	Full sun (mg/kg)
2-Methylfuran	0.0166	0.0233
p-Cresol	0.0055	0.0084
Diacetyl	0.0429	0.0526
2,3-Pentanedione	0.0753	0.0882
Dimethyl Disulphide	0.0025	0.0031
2-Vinylfuran	0.0043	0.0058
Vinylpyrazine	0.0019	0.0022
2,3-Hexanedione	0.0033	0.0048
1-Methylpyrrole	0.0081	0.0097
2,5-Dimethylfuran	0.0021	0.0030
2-Ethyl-3,6-dimethylpyrazine	0.0026	0.0030
2,4,5-Trimethyloxazole	0.0007	0.0008
2-Pentylfuran	0.0002	0.0002
2-Methoxymethylfuran	0.0014	0.0019
2-Methylpyrazine	0.2913	0.3081
Dihydro-2-methyl-3-furanone	0.0458	0.0568
4-Methylthiazole	0.0021	0.0024
2,6-Diethylpyrazine	0.0004	0.0005
2,5-Dimethylpyrazine	0.0313	0.0325
2,6-Dimethylpyrazine	0.0654	0.0670
2-Ethylpyrazine	0.0358	0.0371
2,3-Dimethylpyrazine	0.0090	0.0123
2-Methyl-2-cyclopentenone	0.0013	0.0018
2-Ethyl-6-methylpyrazine	0.0118	0.0136
2-Ethyl-5-methylpyrazine	0.0075	0.0088
2,3,5-Trimethylpyrazine	0.0087	0.0099
2-Ethyl-3-methylpyrazine	0.0070	0.0078
Propylpyrazine	0.0269	0.0276
Acetoin	0.0212	0.0261
Hexanal	0.0005	0.0005
4-Ethylguaiacol	0.0000	0.0001
Pyrrole	0.0077	0.0092
Acetic acid	0.3698	0.3915
Furfural	0.3511	0.4027
Acetoxyacetone	0.0999	0.1368
2-Fufurylmethyl sulfide	0.0007	0.0008
2-Acetylfuran	0.0304	0.0363
2-Ethyl-3,5-dimethylpyrazine	0.0006	0.0007

2,3-Dimethyl-2-cyclopentenone	0.0004	0.0004
Acetoxy-2-butanone	0.0134	0.0190
2-Furfurylacetate	0.0164	0.0226
Propionic acid	0.0097	0.0103
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0541	0.0805
2-Acetylpyridine	0.0005	0.0006
1-Methyl-2-formylpyrrole	0.0021	0.0033
g-Butyrolactone	0.0075	0.0091
Furfuryl alcohol	0.1925	0.2104
Isovaleric acid	0.0241	0.0233
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0056	0.0057
1-Furfurylpyrrole	0.0011	0.0011
2-Methoxy-4-vinylguaiacol	0.0009	0.0011
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0019	0.0019
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0019	0.0023
Pyridine	0.0653	0.0673
Guaiacol	0.0004	0.0005

Table 7 The quantitative data for volatile aroma compounds in the contrast group service shade trees vs timber shade trees

	Service (mg/kg)	Timber (mg/kg)
2-Methylfuran	0.0218	0.0137
p-Cresol	0.0084	0.0054
Diacetyl	0.0567	0.0432
2,3-Pentanedione	0.1003	0.0777
Dimethyl Disulphide	0.0031	0.0019
2-Vinylfuran	0.0061	0.0039
Vinylpyrazine	0.0022	0.0019
2,3-Hexanedione	0.0055	0.0032
1-Methylpyrrole	0.0099	0.0070
2,5-Dimethylfuran	0.0028	0.0017
2-Ethyl-3,6-dimethylpyrazine	0.0026	0.0025
2,4,5-Trimethyloxazole	0.0008	0.0007

2-Pentylfuran	0.0003	0.0002
2-Methoxymethylfuran	0.0017	0.0010
2-Methylpyrazine	0.3163	0.3068
Dihydro-2-methyl-3-furanone	0.0566	0.0415
4-Methylthiazole	0.0025	0.0021
2,6-Diethylpyrazine	0.0004	0.0004
2,5-Dimethylpyrazine	0.0329	0.0315
2,6-Dimethylpyrazine	0.0678	0.0673
2-Ethylpyrazine	0.0376	0.0367
2,3-Dimethylpyrazine	0.0121	0.0117
2-Methyl-2-cyclopentenone	0.0016	0.0010
2-Ethyl-6-methylpyrazine	0.0130	0.0126
2-Ethyl-5-methylpyrazine	0.0084	0.0083
2,3,5-Trimethylpyrazine	0.0097	0.0097
2-Ethyl-3-methylpyrazine	0.0076	0.0078
Propylpyrazine	0.0290	0.0279
Acetoin	0.0269	0.0214
Hexanal	0.0009	0.0004
4-Ethylguaiacol	0.0001	0.0000
Pyrrole	0.0087	0.0075
Acetic acid	0.4053	0.3336
Furfural	0.4159	0.3462
Acetoxyacetone	0.1181	0.0811
2-Fufurylmethyl sulfide	0.0009	0.0005
2-Acetylfuran	0.0358	0.0278
2-Ethyl-3,5-dimethylpyrazine	0.0006	0.0005
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0003
Acetoxy-2-butanone	0.0169	0.0114
2-Furfurylacetate	0.0255	0.0161
Propionic acid	0.0109	0.0080
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0831	0.0587
2-Acetylpyridine	0.0006	0.0004
1-Methyl-2-formylpyrrole	0.0033	0.0023
g-Butyrolactone	0.0095	0.0078
Furfuryl alcohol	0.2074	0.1813
Isovaleric acid	0.0293	0.0265
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0057	0.0056
1-Furfurylpyrrole	0.0012	0.0008
2-Methoxy-4-vinylguaiacol	0.0010	0.0008
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003

2-Acetylpyrrole	0.0018	0.0018
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0021	0.0018
Pyridine	0.0711	0.0480
Guaiacol	0.0005	0.0003

Table 8 The quantitative data for volatile aroma compounds in the contrast group legume timber shade trees vs mom-legume timber shade trees

	Legume (mg/kg)	Non-legume (mg/kg)
2-Methylfuran	0.0200	0.0137
p-Cresol	0.0079	0.0054
Diacetyl	0.0534	0.0432
2,3-Pentanedione	0.0938	0.0777
Dimethyl Disulphide	0.0029	0.0019
2-Vinylfuran	0.0057	0.0039
Vinylpyrazine	0.0022	0.0019
2,3-Hexanedione	0.0052	0.0032
1-Methylpyrrole	0.0098	0.0070
2,5-Dimethylfuran	0.0026	0.0017
2-Ethyl-3,6-dimethylpyrazine	0.0027	0.0024
2,4,5-Trimethyloxazole	0.0009	0.0007
2-Pentylfuran	0.0003	0.0002
2-Methoxymethylfuran	0.0016	0.0010
2-Methylpyrazine	0.3174	0.2918
Dihydro-2-methyl-3-furanone	0.0525	0.0415
4-Methylthiazole	0.0025	0.0021
2,6-Diethylpyrazine	0.0004	0.0004
2,5-Dimethylpyrazine	0.0333	0.0325
2,6-Dimethylpyrazine	0.0694	0.0663
2-Ethylpyrazine	0.0381	0.0347
2,3-Dimethylpyrazine	0.0127	0.0127
2-Methyl-2-cyclopentenone	0.0015	0.0010
2-Ethyl-6-methylpyrazine	0.0133	0.0116
2-Ethyl-5-methylpyrazine	0.0086	0.0079
2,3,5-Trimethylpyrazine	0.0098	0.0087
2-Ethyl-3-methylpyrazine	0.0078	0.0068
Propylpyrazine	0.0281	0.0273
Acetoin	0.0260	0.0214
Hexanal	0.0007	0.0004

4-Ethylguaiacol	0.0001	0.0000
Pyrrole	0.0089	0.0075
Acetic acid	0.3843	0.3336
Furfural	0.3949	0.3462
Acetoxyacetone	0.1153	0.0811
2-Fufurylmethyl sulfide	0.0009	0.0005
2-Acetylfuran	0.0336	0.0278
2-Ethyl-3,5-dimethylpyrazine	0.0006	0.0005
2,3-Dimethyl-2-cyclopentenone	0.0005	0.0003
Acetoxy-2-butanone	0.0167	0.0114
2-Furfurylacetate	0.0243	0.0161
Propionic acid	0.0103	0.0080
3-Methylpyrrole	0.0002	0.0002
5-Methylfurfural	0.0797	0.0587
2-Acetylpyridine	0.0005	0.0004
1-Methyl-2-formylpyrrole	0.0033	0.0023
g-Butyrolactone	0.0093	0.0078
Furfuryl alcohol	0.2043	0.1813
Isovaleric acid	0.0265	0.0257
2-Furfuryl-5-methylfuran	0.0001	0.0001
2,5-Dihydrofuranone	0.0056	0.0056
1-Furfurylpyrrole	0.0011	0.0008
2-Methoxy-4-vinylguaiacol	0.0010	0.0008
Phenylethyl alcohol	0.0002	0.0002
2-Thiophenemethanol	0.0003	0.0003
2-Acetylpyrrole	0.0018	0.0016
Difurfuryl ether	0.0001	0.0001
2-Formylpyrrole	0.0022	0.0018
Pyridine	0.0719	0.0480
Guaiacol	0.0005	0.0003

References

AESCHBACHER, H., WOLLEB, U., LÖLIGER, J., SPADONE, J. & LIARDON, R. 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. *Food and chemical toxicology,* 27**,** 227-232.

AGRESTI, P. D. M., FRANCA, A. S., OLIVEIRA, L. S. & AUGUSTI, R. 2008. Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. *Food chemistry,* 106, 787-796.

ALÈGRE, C. 1959. Climates et caféiers d'Arabie. *Agron. Trop,* 14, 23-58.

AMIN, M., HOSSAIN, M. & ROY, K. 2004. Effects of moisture content on some physical properties of lentil seeds. *Journal of Food Engineering,* 65**,** 83-87.

AMRANI-HEMAIMI, M., CERNY, C. & FAY, L. B. 1995. Mechanisms of formation of alkylpyrazines in the Maillard reaction. *Journal of agricultural and food chemistry,* 43**,** 2818-2822.

ANDERSON, K. A. & SMITH, B. W. 2002. Chemical profiling to differentiate geographic growing origins of coffee. *Journal of agricultural and food chemistry,* 50**,** 2068-2075.

ANDERSON, L. & GIBBS, M. 1962. The biosynthesis of caffeine in the coffee plant. *J Biol Chem,* 237**,** 1941-1944.

ARNAUD, M. J. 1988. The metabolism of coffee constituents. *In:* CLARKE, R. J. & MACRAE, R. (eds.) *Coffee.* Essex: Elsevier Applied Science Publishers.

ASHIHARA, H. & CROZIER, A. 2001. Caffeine: a well known but little mentioned compound in plant science. *Trends in plant science,* 6**,** 407-413.

BACON, C. 2005. Confronting the coffee crisis: can fair trade, organic, and specialty coffees reduce small-scale farmer vulnerability in northern Nicaragua? *World development,* 33, 497-511.

BADE-WEGNER, H., HOLSCHER, W. & VITZTHUM, O. Quantification of 2-methylisoborneol in roasted coffee by GC-MS. COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFE, 1993. ASIC ASSOCIATION SCIENTIFIQUE INTERNATIONALE, 537-537.

BAGGENSTOSS, J., POISSON, L., LUETHI, R., PERREN, R. & ESCHER, F. 2007. Influence of Water Quench Cooling on Degassing and Aroma Stability of Roasted Coffee. *Journal of Agricultural and Food Chemistry,* 55**,** 6685-6691.

BEER, J., MUSCHLER, R., KASS, D. & SOMARRIBA, E. 1997. Shade management in coffee and cacao plantations. *Agroforestry systems,* 38**,** 139-164.

BHUMIRATANA, N., ADHIKARI, K. & CHAMBERS, E. 2011. Evolution of sensory aroma attributes from coffee beans to brewed coffee. *LWT-Food Science and Technology,* 44, 2185-2192.

BLACKMAN, A. & NARANJO, M. A. 2012. Does eco-certification have environmental benefits? Organic coffee in Costa Rica. *Ecological Economics,* 83**,** 58-66.

BLANK, I., DEVAUD, S. & FAY, L. B. 1998. Study on the Formation and Decomposition of the Amadori Compound. *The Maillard reaction in foods and medicine,* 223**,** 43.

BLIGH, E. G. & DYER, W. J. 1959. A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. *Canadian Journal of Biochemistry and Physiology,* 37**,** 911-917.

BOARDMAN, N. T. 1977. Comparative photosynthesis of sun and shade plants. *Annual review of plant physiology,* 28**,** 355-377.

BOATENG, S. A., ZICKERMANN, J. & KORNAHRENS, M. 2006. Poultry manure effect on growth and yield of maize. *West African Journal of Applied Ecology,* 9.

BOREUX, V., VAAST, P., MADAPPA, L. P., CHEPPUDIRA, K. G., GARCIA, C. & GHAZOUL, J. 2016. Agroforestry coffee production increased by native shade trees, irrigation, and liming. *Agronomy for sustainable development,* 36, 1-9.

BOTTAZZI, D., FARINA, S., MILANI, M. & MONTORSI, L. 2012. A numerical approach for the analysis of the coffee roasting process. *Journal of food engineering,* 112**,** 243-252.

BOULTER, D. 1970. Protein synthesis in plants. *Annual review of plant physiology,* 21**,** 91-114.

BRADBURY, A. G. W. & HALLIDAY, D. J. 1990. Chemical structures of green coffee bean polysaccharides. *Journal of Agricultural and Food Chemistry,* 38**,** 389-392.

BRADBURY, A. G. W. & HALLIDAY, D. J. 1987. Polysaccharides in green coffee beans. *ASIC.* Paris, France.
BRADBURY, A. G. W. 2001. 1A: Carbohydrates. In: CLARKE, R. J. & VITZTHUM, O. G. (eds.) *Coffee: Recent Development*. Cornwall: Blackwell Science Ltd..
BRIANDET, R., KEMSLEY, E. K. & WILSON, R. H. 1996. Discrimination of Arabica and Robusta in instant coffee by Fourier transform infrared spectroscopy and chemometrics. *Journal of agricultural and food chemistry,* 44**,** 170-174.
CAMPANHA, M. M., SANTOS, R. H. S., DE FREITAS, G. B., MARTINEZ, H. E. P., GARCIA, S. L. R. & FINGER, F. L. 2004. Growth and yield of coffee plants in agroforestry and monoculture systems in Minas Gerais, Brazil. *Agroforestry Systems,* 63**,** 75-82.
CANNELL, M. 1974. Factors affecting arabica coffee bean size in Kenya. *Journal of Horticultural Science,* 49**,** 65-76.
CARISANO, A. & GARIBOLDI, L. 1964. Gas chromatographic examination of the fatty acids of coffee oil. *Journal of the Science of Food and Agriculture,* 15**,** 619-622.
CARUSO, G., VILLARI, A. & VILLARI, G. Quality characteristics of Fragaria vesca L. fruits influenced by NFT solution EC and shading. South Pacific Soilless Culture Conference-SPSCC 648, 2003. 167-175.
CARVALHO, A. M., MENDES, A. N., CARVALHO, G. R., BOTELHO, C. E., GONÇALVES, F. A. & FERREIRA, A. D. 2011. Correlação entre crescimento e produtividade de cultivares de café em diferentes regiões de Minas Gerais, Brasil. *Pesquisa agropecuária brasileira,* 45**,** 269-275.
CASTRO-TANZI, S., DIETSCH, T., URENA, N., VINDAS, L. & CHANDLER, M. 2012. Analysis of management and site factors to improve the sustainability of smallholder coffee production in Tarrazú, Costa Rica. *Agriculture, ecosystems & environment,* 155**,** 172-181.
CHILIMBA, A. D. C., YOUNG, S. D., BLACK, C. R., MEACHAM, M. C., LAMMEL, J. & BROADLEY, M. R. 2012. Agronomic biofortification of maize with selenium (Se) in Malawi. *Field Crops Research,* 125**,** 118-128.
CILAS, C., MONTAGNON, C. & BAR-HEN, A. 2011. Yield stability in clones of Coffea canephora in the short and medium term: longitudinal data analyses and measures of stability over time. *Tree Genetics & Genomes,* 7**,** 421-429.
CLARKE, R. 1985. Water and mineral contents. *Coffee.* Springer.
CLARKE, R. & VITZTHUM, O. 2008. *Coffee: recent developments*, John Wiley & Sons.
CLARKE, R. J. & MACRAE, R. 1988. *Coffee volume 2: Technology*, Springer Science & Business Media.
COLTRI, P. P., JUNIOR, J. Z., DUBREUIL, V., RAMIREZ, G. M., PINTO, H. S., CORAL, G. & LAZARIM, C. G. 2015. Empirical models to predict LAI and aboveground biomass of Coffea arabica. *Agroforestry systems,* 89**,** 621-636.
CZERNY, M. & GROSCH, W. 2000. Potent odorants of raw Arabica coffee. Their changes during roasting. *Journal of Agricultural and Food Chemistry,* 48**,** 868-872.
CZERNY, M., MAYER, F. & GROSCH, W. 1999. Sensory study on the character impact odorants of roasted Arabica coffee. *Journal of Agricultural and Food Chemistry,* 47**,** 695-699.
DAMATTA, F. M. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field crops research,* 86**,** 99-114.
DAMATTA, F. M., RONCHI, C. P., MAESTRI, M. & BARROS, R. S. 2007. Ecophysiology of coffee growth and production. *Brazilian Journal of Plant Physiology,* 19**,** 485-510.
DART, S. & NURSTEN, H. 1985. Volatile components. *Coffee.* Springer.
DE CARVALHO GOMES, L., CARDOSO, I. M., DE SÁ MENDONÇA, E., FERNANDES, R. B. A., LOPES, V. S. & OLIVEIRA, T. S. 2016. Trees modify the dynamics of soil CO 2 efflux in coffee agroforestry systems. *Agricultural and Forest Meteorology,* 224**,** 30-39.
DUTRA, E., OLIVEIRA, L., FRANCA, A., FERRAZ, V. & AFONSO, R. 2001. A preliminary study on the feasibility of using the composition of coffee roasting exhaust gas for the determination of the degree of roast. *Journal of Food Engineering,* 47**,** 241-246.
EGGERS, R. & PIETSCH, A. 2001. Technology I: roasting. *Coffee: recent developments*, 90-107.

ELEVITCH, C., IDOL, T., FRIDAY, J., LEPCZYK, C., EASTON SMITH, V. & NELSON, S. 2014. Shade-Grown Coffee in Hawai ʻi: results of a twelve farm study in Kona. Permanent Agriculture Resources, Holualoa, Hawai ʻi.

FARAH, A. 2012. Coffee constituents. *Coffee: emerging health effects and disease prevention*, 1**,** 22-58.

FISK, I. D., GKATZIONIS, K., LAD, M., DODD, C. E. R. & GRAY, D. A. 2009. Gamma-irradiation as a method of microbiological control, and its impact on the oxidative labile lipid component of Cannabis sativa and Helianthus annus. *European Food Research and Technology*, 228**,** 613-621.

FISK, I. D., KETTLE, A., HOFMEISTER, S., VIRDIE, A. & KENNY, J. S. 2012. Discrimination of roast and ground coffee aroma. *Flavour*, 1, 14.

FOLSTAR, P. 1976. *The composition of wax and oil in green coffee beans*, Pudoc.

FRANCA, A. S., OLIVEIRA, L. S., MENDONÇA, J. C. F. & SILVA, X. A. 2005. Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry*, 90**,** 89-94.

FRANCA, A. S., OLIVEIRA, L. S., OLIVEIRA, R. C., AGRESTI, P. C. M. & AUGUSTI, R. 2009. A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. *Journal of Food Engineering*, 92, 345-352.

FREITAS, A. C. & MOSCA, A. 1999. Coffee geographic origin—an aid to coffee differentiation. *Food Research International*, 32, 565-573.

FRISCHKNECHT, P. M. & BAUMANN, T. W. 1985. Stress induced formation of purine alkaloids in plant tissue culture of Coffea arabica. *Phytochemistry*, 24**,** 2255-2257.

GINZ, M., BALZER, H. H., BRADBURY, A. G. & MAIER, H. G. 2000. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. *European Food Research and Technology*, 211**,** 404-410.

GLOESS, A. N., VIETRI, A., WIELAND, F., SMRKE, S., SCHÖNBÄCHLER, B., LÓPEZ, J. A. S., PETROZZI, S., BONGERS, S., KOZIOROWSKI, T. & YERETZIAN, C. 2014. Evidence of different flavour formation dynamics by roasting coffee from different origins: On-line analysis with PTR-ToF-MS. *International Journal of Mass Spectrometry*, 365**,** 324-337.

GREMBECKA, M., MALINOWSKA, E. & SZEFER, P. 2007. Differentiation of market coffee and its infusions in view of their mineral composition. *Science of the Total Environment*, 383**,** 59-69.

GROSCH, W. 2001. Chemistry III: volatile compounds. *Coffee: recent developments***,** 68-89.

HAGGAR, J., BARRIOS, M., BOLAÑOS, M., MERLO, M., MORAGA, P., MUNGUIA, R., PONCE, A., ROMERO, S., SOTO, G. & STAVER, C. 2011. Coffee agroecosystem performance under full sun, shade, conventional and organic management regimes in Central America. *Agroforestry Systems*, 82**,** 285-301.

HO, C., HWANG, H., YU, T. & ZHANG, J. An overview of the Maillard reactions related to aroma generation in coffee. COLLOQUE Scientifique International sur le Café, 15. Montpellier (Francia), Juin 6-11, 1993.. 1993.

HOLSCHER, W. & STEINHART, H. 1994. Formation pathways for primary roasted coffee aroma compounds. ACS Publications.

HOLSCHER, W. & STEINHART, H. 1995. Aroma compounds in green coffee. *Developments in food science*, 37, 785-803.

HOMMA, S. 2001. Chemistry II: Non-Volatile Compounds, Part II. *Coffee: recent developments***,** 50-67.

HU, Y. & SCHMIDHALTER, U. 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *Journal of Plant Nutrition and Soil Science*, 168**,** 541-549.

ISMAIL, I., ANUAR, M. & SHAMSUDIN, R. 2013. Effect on the physico-chemical properties of liberica green coffee beans under ambient storage. *International food research journal*, 20.

KORNMAN, C. 2016. *Green Coffee Analytics Part IV - Density* [Online]. Available: https://royalcoffee.com/green-coffee-analytics-part-iv-density/ [Accessed 13 Sep 2017].

KUCEY, R. & PAUL, E. A. 1981. Carbon flow in plant microbial associations.

KY, C.-L., DOULBEAU, S., GUYOT, B., AKAFFOU, S., CHARRIER, A., HAMON, S., LOUARN, J. & NOIROT, M. 2000. Inheritance of coffee bean sucrose content in the interspecific cross Coffea pseudozanguebariae^{x} Coffea liberica 'dewevrei'. *Plant Breeding*, 119**,** 165-168.

LEMMON, P. E. 1956. A spherical densiometer for estimating forest overstory density. *Forest Sci,* 2**,** 314-320.

LIN, B. B. 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. *Agricultural and Forest Meteorology,* 144**,** 85-94.

LIU, Y. & KITTS, D. D. 2011. Confirmation that the Maillard reaction is the principle contributor to the antioxidant capacity of coffee brews. *Food Research International,* 44, 2418-2424.

LUDWIG, E., RACZEK, N. & KURZROCK, T. Contribution to composition and reactivity of coffee protein. COLLOQUE Scientifique International sur le Café, 16. Kyoto (Japón), Avril 9-14, 1995.. 1995.

LYNCH, J. & LÄUCHLI, A. 1984. Potassium transport in salt-stressed barley roots. *Planta,* 161**,** 295-301.

LYNGBAEK, A. E. & MUSCHLER, R. G. 2001. Productivity and profitability of multistrata organic versus conventional coffee farms in Costa Rica. *Agroforestry systems,* 53**,** 205-213.

MACRAE, R. 1985. Nitrogenous components. *Coffee.* Springer.

MAESTRI, M., BARROS, R. & RENA, A. 2001. *Coffee.* In: Last FT ed. Amsterdam: Elsevier Publishers.

MAIER, H. G. 1981. *Kaffee,* Parey.

MAILLARD, L. C. 1912. Action des acidesamines sur les sucres: formation des melanoidines par voie methodique. *CR Acad. Sci.(Paris),* 154**,** 66-68.

MARTíN, M. A. J., PABLOS, F. & GONZÁLEZ, A. G. 1998. Discrimination between arabica and robusta green coffee varieties according to their chemical composition. *Talanta,* 46, 1259-1264.

MARTÍNEZ-BALLESTA, M., DOMINGUEZ-PERLES, R., MORENO, D., MURIES, B., ALCARAZ-LÓPEZ, C., BASTÍAS, E., GARCÍA-VIGUERA, C. & CARVAJAL, M. 2010. Minerals in plant food: effect of agricultural practices and role in human health. A review. *Agronomy for Sustainable Development,* 30**,** 295-309.

MCCUSKER, R. R., GOLDBERGER, B. A. & CONE, E. J. 2003. Caffeine Content of Specialty Coffees. *Journal of Analytical Toxicology,* 27**,** 520-522.

MCDOWELL, L. R. 1992. *Minerals in animal and human nutrition,* Academic Press Inc.

M'ITUNGO, A. & VAN DER VOSSEN, H. 1981. Nutrient requirements of coffee seedlings in polybag nurseries: the effect of foliar feeds in relation to type of potting mixture. *Kenya Coffee,* 46, 181-7.

MITCHELL, H. 1988. Cultivation and harvesting of the arabica coffee tree. *Coffee, Agronomy,* 4, 43-90.

MONTANARO, G., DICHIO, B., XILOYANNIS, C. & CELANO, G. 2006. Light influences transpiration and calcium accumulation in fruit of kiwifruit plants (Actinidia deliciosa var. deliciosa). *Plant Science,* 170**,** 520-527.

MONTAGNON, C., GUYOT, B., CILAS, C. & LEROY, T. 1998. Genetic parameters of several biochemical compounds from green coffee, Coffea canephora. *Plant breeding,* 117, 576-578.

MONTAVON, P., MAURON, A.-F. & DURUZ, E. 2003. Changes in green coffee protein profiles during roasting. *Journal of agricultural and food chemistry,* 51, 2335-2343.

MORAIS, H., MARUR, C. J., CARAMORI, P. H., DE ARRUDA RIBEIRO, A. M. & GOMES, J. C. 2003. Características fisiológicas e de crescimento de cafeeiro sombreado com guandu e cultivado a pleno sol. *Pesquisa Agropecuária Brasileira,* 38**,** 1131-1137.

MOREIRA, A. S., NUNES, F. M., DOMINGUES, M. R. & COIMBRA, M. A. 2012. Coffee melanoidins: structures, mechanisms of formation and potential health impacts. *Food & function,* 3, 903-915.

MÜLLER, K. & HIPPE, J. 1987. Influence of differences in nutrition on important quality characteristics of some agricultural crops. *Plant and Soil,* 100, 35-45.

MUSCHLER, R. G. 2001. Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. *Agroforestry Systems,* 51**,** 131-139.

NABRZYSKI, M. 2007. Functional role of some minerals in foods. *Mineral components in foods***,** 363-388.

NANTIYAKUL, N., FURSE, S., FISK, I., FOSTER, T. J., TUCKER, G. & GRAY, D. A. 2012. Phytochemical Composition of Oryza sativa (Rice) Bran Oil Bodies in Crude and Purified Isolates. *Journal of the American Oil Chemists' Society*, 89**,** 1867-1872.

NANTIYAKUL, N., FURSE, S., FISK, I. D., TUCKER, G. & GRAY, D. A. 2013. Isolation and characterization of oil bodies from Oryza sativa bran and studies of their physical properties. *Journal of Cereal Science,* 57**,** 141-145.

NIJSSEN, L., VISSCHER, C., MAARSE, H., WILLEMSENS, L. & BOELENS, M. 1996. Volatile compounds in food: qualitative and quantitative data. *Central Institute for Nutrition and Food Research, TNO: Zeist, The Netherlands*.

NITIKA, PUNIA, D. & KHETARPAUL, N. 2008. Physico-chemical characteristics, nutrient composition and consumer acceptability of wheat varieties grown under organic and inorganic farming conditions. *International journal of food sciences and nutrition*, 59, 224-245.

NOPONEN, M. R., EDWARDS-JONES, G., HAGGAR, J. P., SOTO, G., ATTARZADEH, N. & HEALEY, J. R. 2012. Greenhouse gas emissions in coffee grown with differing input levels under conventional and organic management. *Agriculture, ecosystems & environment,* 151**,** 6-15.

NOPONEN, M. R., HAGGAR, J. P., EDWARDS-JONES, G. & HEALEY, J. R. 2013. Intensification of coffee systems can increase the effectiveness of REDD mechanisms. *Agricultural systems,* 119**,** 1-9.

NYGREN, P., FERNÁNDEZ, M. P., HARMAND, J.-M. & LEBLANC, H. A. 2012. Symbiotic dinitrogen fixation by trees: an underestimated resource in agroforestry systems? *Nutrient cycling in agroecosystems,* 94**,** 123-160.

OGITA, S., UEFUJI, H., MORIMOTO, M. & SANO, H. 2004. Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. *Plant molecular biology,* 54**,** 931-941.

OHLROGGE, J. & BROWSE, J. 1995. Lipid Biosynthesis. *The Plant Cell*, 7**,** 957-970.

OLIVEIRA, M., CASAL, S., MORAIS, S., ALVES, C., DIAS, F., RAMOS, S., MENDES, E., DELERUE-MATOS, C. & OLIVEIRA, M. B. P. 2012. Intra-and interspecific mineral composition variability of commercial instant coffees and coffee substitutes: Contribution to mineral intake. *Food chemistry,* 130**,** 702-709.

OLIVEIRA, M., RAMOS, S., DELERUE-MATOS, C. & MORAIS, S. 2015. Espresso beverages of pure origin coffee: Mineral characterization, contribution for mineral intake and geographical discrimination. *Food chemistry,* 177**,** 330-338.

OLSEN, S. & WATANABE, F. 1979. Interaction of added gypsum in alkaline soils with uptake of iron, molybdenum, manganese, and zinc by sorghum. *Soil Science Society of America Journal,* 43**,** 125-130.

PARLIAMENT, T. H., MCGORRIN, R. J., MORELLO, M. J. & PARLIAMENT, T. H. 1994. *Thermally Generated Flavors: Maillard, Microwave, and Extrusion Processes: Developed from a Symposium Sponsored by the Division of Agricultural and Food Chemistry at the 204th National Meeting of the American Chemical Society, Washington, DC, August 23-28, 1992*, American Chemical Society.

PERRONE, D., DONANGELO, C. M. & FARAH, A. 2008. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry. *Food Chemistry,* 110, 1030-1035.

PERRONE, D., FARAH, A., DONANGELO, C. M., DE PAULIS, T. & MARTIN, P. R. 2008. Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. *Food Chemistry*, 106, 859-867.

POTTS, J., LYNCH, M., WILKINGS, A., HUPPÉ, G. A., CUNNINGHAM, M. & VOORA, V. A. 2014. *The state of sustainability initiatives review 2014: Standards and the green economy*, International Institute for Sustainable Development Winnepeg.

PRADHAN, R., NAIK, S., BHATNAGAR, N. & SWAIN, S. 2008. Moisture-dependent physical properties of Karanja (Pongamia pinnata) kernel. *Industrial Crops and Products,* 28**,** 155-161.

REINECCIUS, G. The Maillard reaction and coffee flavor. COLLOQUE Scientifique International sur le Café, 16. Kyoto (Japón), Avril 9-14, 1995.. 1995.

RISTICEVIC, S., CARASEK, E. & PAWLISZYN, J. 2008. Headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric methodology for geographical origin verification of coffee. *Analytica chimica acta*, 617, 72-84.

ROBARTS, R. D. & ZOHARY, T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research,* 21**,** 391-399.

ROGERS, W., BEZARD, G., DESHAYES, A., PETIARD, V. & MARRACCINI, P. An 11S-Type Storage Protein from Coffea arabica L. Endosperm: Biochemical Characterization, Promoter Function and Expression During Grain Maturation. COLLOQUE Scientifique International sur le Café, 17. Nairobi (Kenya), Juillet 20-25, 1997.. 1997.

SAEMAN, J. F. 1945. Kinetics of wood saccharification-hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. *Industrial & Engineering Chemistry,* 37, 43-52.

SCHENKER, S., HEINEMANN, C., HUBER, M., POMPIZZI, R., PERREN, R. & ESCHER, R. 2002. Impact of roasting conditions on the formation of aroma compounds in coffee beans. *Journal of food science,* 67**,** 60-66.

SCHWARTZBERG, H. 2002. Modelling bean heating during batch roasting of coffee beans. *Engineering and Food for the 21st Century,* 1036.

SEUFERT, V., RAMANKUTTY, N. & FOLEY, J. A. 2012. Comparing the yields of organic and conventional agriculture. *Nature,* 485**,** 229-232.

SEMMELROCH, P. & GROSCH, W. 1996. Studies on character impact odorants of coffee brews. *Journal of Agricultural and Food Chemistry,* 44, 537-543.

SILVA, E. A. D., MAZZAFERA, P., BRUNINI, O., SAKAI, E., ARRUDA, F. B., MATTOSO, L. H. C., CARVALHO, C. R. & PIRES, R. C. M. 2005. The influence of water management and environmental conditions on the chemical composition and beverage quality of coffee beans. *Brazilian Journal of Plant Physiology,* 17, 229-238.

SILWAR, R. & LÜLLMAN, C. 1988. The determination of mono-and disaccharides in green arabica and robusta coffees using high performance liquid chromatography. *Café, Cacao, Thè,* 32**,** 319-322.

SIMS, J. T. 1986. Soil pH effects on the distribution and plant availability of manganese, copper, and zinc. *Soil Science Society of America Journal,* 50**,** 367-373.

SINGH, P. C., SINGH, R. K., BHAMIDIPATI, S., SINGH, S. N. & BARONE, P. 1997. Thermophysical properties of fresh and roasted coffee powders. *Journal of food process engineering,* 20**,** 31-50.

SIVETZ, M. & DESROSIER, N. W. Coffee technology. 1979. Avi Westport, Connecticut.

SPEER, K. & KÖLLING-SPEER, I. 2001. 1C Lipids. *In:* CLARKE, R. J. & VITZTHUM, O. G. (eds.) *Coffee Recent development.* Cornwall: Blackwell Science Ltd.

SPEER, K. & KÖLLING-SPEER, I. 2006. The lipid fraction of the coffee bean. *Brazilian Journal of Plant Physiology,* 18**,** 201-216.

STARCK, Z., SIWIEC, A. & CHOTUJ, D. 1994. Distribution of calcium in tomato plants in response to heat stress and plant growth regulators. *Plant and Soil,* 167**,** 143-148.

STUMPF, W., CONN, P. M. & PREISS, J. 2012. *The biochemistry of plants: Carbohydrates*, Academic Press.

TAUGOURDEAU, S., LE MAIRE, G., AVELINO, J., JONES, J. R., RAMIREZ, L. G., QUESADA, M. J., CHARBONNIER, F., GÓMEZ-DELGADO, F., HARMAND, J.-M. & RAPIDEL, B. 2014. Leaf area index as an indicator of ecosystem services and management practices: an application for coffee agroforestry. *Agriculture, ecosystems & environment,* 192**,** 19-37.

TRESSL, R. 1989. Formation of flavor components in roasted coffee. ACS Publications.

TRESSL, R., WONDRAK, G. T., GARBE, L.-A., KRÜGER, R.-P. & REWICKI, D. 1998. Pentoses and hexoses as sources of new melanoidin-like Maillard polymers. *Journal of Agricultural and Food Chemistry,* 46**,** 1765-1776.

TROMP, J. 1975. The effect of temperature on growth and mineral nutrition of fruits of apple, with special reference to calcium. *Physiologia Plantarum,* 33**,** 87-93.

TRUGO, L. C. & MACRAE, R. 1985. The use of the mass detector for sugar analysis of coffee products. *ASIC.* Paris, France.

TSCHARNTKE, T., CLOUGH, Y., BHAGWAT, S. A., BUCHORI, D., FAUST, H., HERTEL, D., HÖLSCHER, D., JUHRBANDT, J., KESSLER, M. & PERFECTO, I. 2011. Multifunctional shade-tree management in tropical agroforestry landscapes–a review. *Journal of Applied Ecology,* 48**,** 619-629.

TULLY, K. L. & LAWRENCE, D. 2011. Closing the loop: nutrient balances in organic and conventional coffee agroforests. *Journal of sustainable agriculture,* 35**,** 671-695.

TULLY, K. L., LAWRENCE, D. & SCANLON, T. M. 2012. More trees less loss: Nitrogen leaching losses decrease with increasing biomass in coffee agroforests. *Agriculture, ecosystems & environment,* 161**,** 137-144.

VAAST, P., BERTRAND, B., PERRIOT, J. J., GUYOT, B. & GENARD, M. 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (Coffea arabica L.) under optimal conditions. *Journal of the Science of Food and Agriculture,* 86**,** 197-204.

VAAST, P., VAN KANTEN, R., SILES, P., DZIB, B., FRANCK, N., HARMAND, J.-M. & GÉNARD, M. Shade: a key factor for coffee sustainability and quality. 2005. ASIC.

VALKILA, J. 2009. Fair Trade organic coffee production in Nicaragua — Sustainable development or a poverty trap? *Ecological Economics,* 68**,** 3018-3025.

VAN DER VOSSEN, H. A. 2001. Agronomy I: coffee breeding practices. *Coffee: recent developments***,** 184-201.

WAJDA, P. & WALCZYK, D. 1978. Relationship between acid value of extracted fatty matter and age of green coffee beans. *Journal of the Science of Food and Agriculture,* 29**,** 377-380.

WILLSON, C. 1985. Mineral nutrition and fertiliser needs. *Coffee*. Springer.

WILSON, A., PETRACCO, M. & ILLY, E. Some preliminary investigations of oil biosynthesis in the coffee fruit and its subsequent re-distribution within green and roasted beans. Proceedings of the 17th International Conference on Coffee Science, 92, 1997.

YANG, N., LIU, C., LIU, X., DEGN, T. K., MUNCHOW, M. & FISK, I. 2016. Determination of volatile marker compounds of common coffee roast defects. Food Chemistry, 211, 206-214.

YERETZIAN, C., JORDAN, A. & LINDINGER, W. 2003. Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. *International Journal of Mass Spectrometry*, 223, 115-139.