Examining growth, yield and bean quality of Ethiopian coffee trees: towards optimizing resources and tree management

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Abstract

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Coffee (*Coffea arabica* L.) bean production and quality are determined by a diversity of interacting factors (e.g. shade, nitrogen, crop traits). Bean yield increases with increase in radiation, but adequate fertilizer supplies are needed to sustain the productivity. This thesis analysed coffee tree growth, bean production and bean quality in relation to different degrees of exposure to radiation and nitrogen supply. Growth of leaves and branches and properties of leaves such as specific leaf area, nitrogen content per unit leaf area and light-saturated rate of photosynthesis were determined. Radiation interception and nitrogen uptake were also determined as were radiation use efficiency and apparent nitrogen recovery. Tree biomass and coffee bean yield responded positively to both radiation and nitrogen supply. Abundant bean yield to the detriment of vegetative growth, however, resulted in biennial bearing in coffee trees. Effects of fruit load on coffee tree growth and productivity were studied for two consecutive years and the result showed that competition between fruit growth and vegetative growth predisposed the trees for biennial bearing. Reduced vegetative growth when fruit load is high reduces the number of flower bearing nodes and hence yields in the next season. Coffee quality is a sum of favourable characteristics that satisfies requirements of different actors in the coffee chain and is the factor determining the price on the coffee market. This study has also examined coffee quality attributes in relation to radiation and nitrogen, fruit load manipulation, and genotype by environment (different altitudes) interactions. The result indicated that factors and conditions that support non-limiting supply of resources for bean to grow and a sufficient long period of maturation promote coffee bean quality. Overall, the study gained further understanding of coffee tree growth, yield and bean quality responses to aforementioned factors and explored traits that underlie the patterns. Further works are required to use the traits and describe the behaviour of coffee trees in different agro-ecosystems.

Contents

Chapter 1

General introduction

General introduction

Population and environment

The world population has grown considerably since 1950, going from 2.5 billion to 6.2 billion in 2000 and reaching 7 billion in 2012 (Teshome 2014). Currently it still grows at an annual rate of about 1.2% per year (adding about 80 million per year to the planet) and it is expected to reach 9 - 10 billion by the end of the century (Cuffaro 2001; UNESCO 2004). This projected increase in global population will primarily take place in developing countries, increasing food demand and putting already vulnerable agro-ecosystems under further pressure (Bielli et al. 2001; Cuffaro 2001). The population-environment related problems are particularly acute in Ethiopia (Cuffaro 2001) where the population has risen from about 53 million in 1994 to 71 million in 2004 (a 33% rise), and by 2015**,** the total population was projected to become about 94 million 76 million of which live in rural areas and depend on agriculture for part of their livelihood (CSA 2012).

About 66% of the country's land is considered suitable for agriculture (UNESCO 2004). But the above mentioned population growth has increased intensity of use with environmental effects including: deforestation, erosion and land degradation (Bielli et al. 2001; Teshome 2014). Increased pressure on land has two risks (i) unsustainable types of land use, resulting in low yields and soil degradation and (ii) destruction of vulnerable, unique natural habitats resulting in a non-reversible loss of biodiversity. Both effects are occurring in Ethiopia in a wide scale. For instance, deforestation in Ethiopia has been severe and most of Ethiopia's last remaining forests that retain genetically diverse wild Arabica coffee populations are under threat of land conversion (Ahrends et al. 2010; Gole et al. 2008; Tadesse et al. 2014). There is thus an urgent need for strategies to sustainably increase agricultural production, particularly of small-scale subsistence farms, without adversely affecting the environment and coffee genetic resources of the country.

The coffee tree

Coffee belongs to the family Rubiaceae and the genus Coffea (Cannell 1985; Wintgens 2009), in which there are more than 70 species. However, only two of these species are commercially used: *Coffea arabica* L (Arabica), providing 75% and *Coffea canephora Pierre ex A. Froehner* (Robusta), providing 25% of world's production (Belitz et al. 2009; Davis et al. 2012). Globally, coffee is the second most traded commodity after oil (Davis et al. 2012;

Esquivel and Jiménez 2012). *C. arabica* is allotetraploid (2n = 4x = 44) species and has its primary centre of origin and diversity in the southwestern Ethiopian highlands. *C. canephora* is a cross pollinating diploid (2n = 2x = 22) species and is more widely dispersed in tropical Africa (Coste et al. 1992; Ky et al. 2001; Leroy et al. 2006).

Under cultivation, *C. arabica* thrives in tropical highlands, mainly in Latin America, East Africa and India, at altitudes between 1300 – 2800 m with an optimum mean daily temperature in the range of 18 °C to 22 °C (Jha et al. 2014; Wintgens 2009). *C. canephora* thrives in tropical lowlands of West Africa, parts of East Africa (e.g. Uganda), Indonesia, Vietnam and Brazil, commonly at altitudes below 1000 m with mean daily temperature in the range of 24 °C to 26 °C (Ky et al. 2001; Wintgens 2009). *C. arabica* is characterized by its lower bitterness, lower caffeine and better flavour, sweeter taste with an aromatic fragrance than Robusta. It is more appreciated by consumers and is sold at distinctly higher price than Robusta (Keidel et al. 2010; Ky et al. 2001; Leroy et al. 2006; Romano et al. 2014). Conversely, Robusta produces beans with strong and cocoa flavours (Cagliani et al. 2013) and it can grow in places that the Arabica will not; it is less susceptible to diseases and pests (Miyanari 2008). As *C. arabica* L. is the only Ethiopia's export coffee, this thesis concerns this species.

Morphology

C arabica L. is a woody perennial evergreen dicotyledonous tree or shrub of variable size growing up to 8-10 m high (Cambrony 1992; Coste et al. 1992; Wrigley 1995). A well-known feature of coffee is the existence of two types of branches: orthotropic, commonly called suckers, which grow vertically, and plagiotropic branches, which grow horizontally. The first levels of plagiotropic branches, inserted on an orthotropic parent are called primaries. Primary branches generate from the "head of series" buds (buds located in the leaf axil, Fig. 1) and give rise to secondary branches, which, in turn, give rise to tertiary and quaternary ramifications (Wintgens 2009).

Branch growth is a typical form of monopodial branching where the primaries remain subsidiary to the main stem, which continues to grow indefinitely by extension of the apical buds (Wintgens 2009; Wrigley 1995). In *C. arabica*, active growth of the branches starts with the onset of the rains (DaMatta et al. 1999; Rena et al. 1994). In countries like Viçosa, Brazil coffee trees growth is slow during the dry cool season and is rapid in the rainy warm season (Silva et al. 2004).

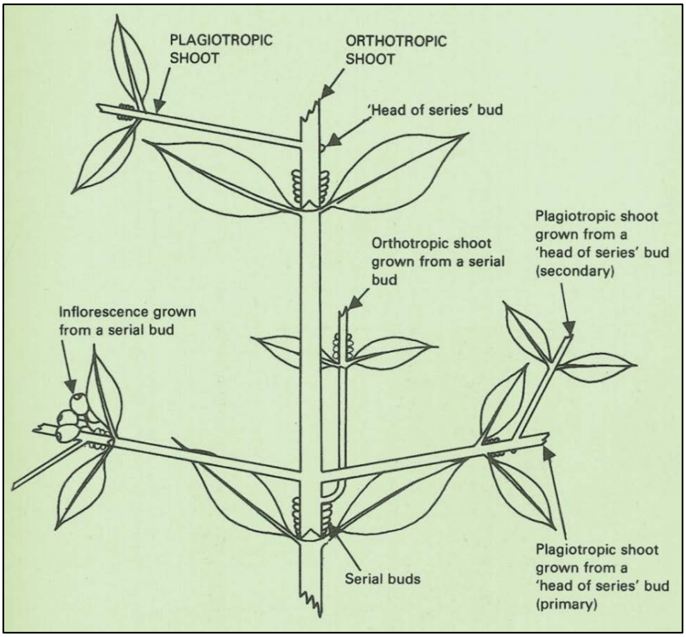

Fig. 1. Shoots and buds of a coffee plant: adapted from Cannell (1985)

The branch apex initiates additional phytomers: units consisting of leaves, node, internode and lateral buds. Coffee leaves grow in opposite pairs along the main stem and branches (Wintgens 2009) and leaf size increases with moderate shade (Rena et al. 1994). On plagiotropic branches, buds develop into flower clusters or grow out into lateral branches (Cannell 1985). Flowering occurs at some distance from the apex. On a branch, the zone with flowers and fruits moves in apical direction; at tree level, flowering and fruiting move from lower to higher branches. Flowering is triggered by rain or temperature drop (Rena et al. 1994). In a zone with buds 'ready to flower' fractions of flower buds may open sequentially upon several inductive events (DaMatta et al. 2007). After three years, coffee tree reaches full maturity and begins to yield a normal crop (Wintgens 2009).

Physiology

C. arabica L. grows naturally in shaded habitats and exhibits some physiological and structural characteristics of shade plants (e.g. low rate of photosynthesis) (Ramalho et al. 1997; Van der Vossen 2005). However, recent studies showed that coffee plants have ability to acclimate to sunlight and produce higher yields without shade, at least if adequate amounts of plant nutrients are supplied (DaMatta 2004; Fahl et al. 1994; Pompelli et al. 2010). The net rate of light saturated CO_2 assimilation, A_{max}, typically ranges between 4 –11 μmolm^{-2} s^{-1} (DaMatta et al. 2007). These values are low compared to many other tropical tree crops (e.g. Ochroma spp., 5 – 25 μmol m^{-2} s^{-1}) but are comparable to other shade tolerant tree species (Poorter and Bongers 2006; Selaya and Anten 2010). Maximum photosynthetic rate per unit nitrogen per leaf area in coffee leaves is also relatively low compared to other C_3 species (Evans 1989; Vos and van der Putten 1998). Growing evidences suggest that the low rates of A_{max} in coffee would be linked to low stomatal diffusive conductance (Araujo et al. 2008; DaMatta et al. 2002; Ronchi et al. 2006).

C. arabica L. is prone to biennial bearing (Cannell 1985; DaMatta 2004), i.e., the tendency to produce large fruit loads in one year followed by low production in the next. Biennial bearing is a considerable problem in coffee as such fluctuations in production result in unstable incomes to farmers. The low crop in the "off" year is directly related to the failure of the plant to produce the preceding season an adequate number of nodes with flower buds (Cannell 1985). The manifestation of biennial bearing is more severe in open sun than in shaded systems (DaMatta 2004).

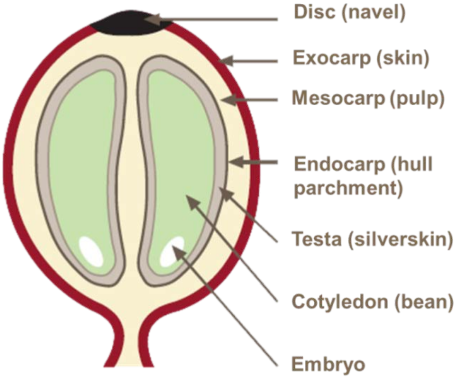

Fig. 2. Cross-sections of coffee cherry: adapted from Yara (2015)

Bean yield and chemical compositions

The fruit of the coffee tree is known as a berry and the beans, which develop inside the berry, are used as the basic element for producing coffee liquor (Wintgens 2009). The coffee fruit growth pattern follows either a double sigmoid-shaped (DaMatta et al. 2007; Geromel et al. 2006) or linear curve (Clowes and Wilson 1977). Each coffee fruit contains two beans covered by a silver skin and parchment layers and further surrounded by pulp (Fig. 2). Coffee yield is generally measured as fresh berry weight per plant or per plot and converted into green coffee weight by applying a conversion ratio (Wintgens 2009). Arabica coffee can potentially produce 90 to 480 g dry green beans per tree (Bruno et al. 2011; Campanha et al. 2004; Haggar et al. 2011; Lin 2009; Obso 2006; Peeters et al. 2003).

Caffeine is the most known component of coffee beans and in raw Arabica coffee the values vary between 0.8 and 1.4% (w/w)(Mussatto et al. 2011). Coffee bean also contains components like cellulose, minerals, sugars, lipids, tannin and polyphenols (Belitz et al. 2009; Grembecka et al. 2007; Mussatto et al. 2011; Santos et al. 2012). Chlorogenic acid, a compound that is able to reduce blood sugar levels and potentially exert an anti-diabetic effect (Meng et al. 2013) is another important constituent of coffee bean. Its proportions may vary from 7 to 12% (w/w) (Belitz et al. 2009; Trugo et al. 1991) and is three to five times more than caffeine percentage (Mussatto et al. 2011). The quality of coffee beans used to make coffee is directly related to their chemical composition (Wei et al. 2013). For example, coffee flavour and aroma are the result of the presence of volatile constituents and the action of enzymes on these constituents (Clemente et al. 2015).

Coffee production in Ethiopia

C. arabica L. plays an exclusive role in Ethiopia coffee economy and the country is the fifth largest global exporter (ICO 2013). Coffee accounts ca. 33% of Ethiopia's total export earnings and sustains the livelihoods of more than one million coffee growing households (Davis et al. 2012; Negussie et al. 2007). Based on differences in intensity of management, four major coffee production systems are commonly distinguished: forest, semi-forest, garden and plantation system (Labouisse et al. 2008; Mekuria et al. 2004; Petit 2007). In forest production systems, wild Arabica coffee is managed inside the forest by removing competing under growth vegetation and some canopy trees (Demel 1999; Wiersum et al. 2008). In semi-forest coffee systems, farmers transplant semi-wild coffee plants that regenerate spontaneously inside the forest to fill open spaces. Both systems account for 5 and 35% of national coffee productions, respectively (Labouisse et al. 2008; Petit 2007). In garden production systems, coffee is characteristically grown in homesteads i.e. close to the house under a few shade trees usually combined with other crops and fruits. Plantation coffee production takes place on cleared land and is managed to maximize the volume of production and productivity. The former accounts for 50% while the latter for 10% of the national coffee production system (Hundera et al. 2013; Labouisse et al. 2008).

Coffee bean processing

There are two widely applied methods of coffee processing, namely dry and wet processing. In the dry processing method (or unwashed sun-dried coffee), the handpicked berries are dried on mats, concrete, or cement floors immediately after they have been picked. After drying to a moisture content of about 11.5 per cent, the outer layer of the berries are removed by hulling and the green bean obtained is ready for marketing. In the wet method (or washed coffee), the handpicking mature berries are mechanical depulped and then fermented for approximately 24 - 72 h to remove the mucilage layer (Labouisse et al. 2008; Silva et al. 2005). More than 80% coffee produced in Ethiopia is processed by the dry method of coffee processing (Silva et al. 2008; Wintgens 2009). Dry processing method maintains the inherent chemical contents (e.g. glucose, fructose) of coffee better than wet processing methods (Knopp et al. 2006).

Coffee bean quality

Quality is an important attribute of coffee and it is currently becoming even more important as there is strong competition on the world market (ICO 2015). Production of high quality coffee beans procures a decisive competitive advantage in the markets where competition is strong (Decazy et al. 2003). Ethiopia is naturally endowed with a suitable climate for coffee production and has the potential to produce large amount of differentiated high quality coffee (Labouisse et al. 2008). However, the country's coffee industry is generally characterized by low productivity (about 0.7 ton ha^{-1} of dry green beans) and low quality (Alemayehu et al. 2008; Labouisse et al. 2008). In the mid-1900's, the Ethiopian coffee sector was hit hard and suffered one of the worst economic crises for years (Labouisse et al. 2008; Petit 2007). Producers were poorly competing on the world market, discouraged from continuing to invest labour in their coffee production and shifted to the production of food crops and crops like chat (Amsalu and Ludi 2010). Since liberalization of the world market (e.g. termination of quota system) and changes in supply have resulted in declined coffee earnings and enhanced poverty of smallholder producers. Hence, a general increase in coffee quality is seen as an effective way to strengthen the position and price of Ethiopian coffee on the world market. This requires a systematic analysis of impacts of practices and procedures on Ethiopian coffee quality throughout the production chain, from farm to the point of delivery of green beans to the exporters.

Coffee quality consists of physical attributes, organoleptic characteristics and biochemical composition (Agwanda et al. 2003; Behailu et al. 2008; Fox et al. 2013; Giomo et al. 2012; Joët et al. 2010; Lara-Estrada and Vaast 2007). Physical quality attributes of coffees include size and weight of coffee beans. Organoleptic characteristics are attributes of coffee that can be distinguished organoleptically by professional coffee tasters, based on established terminologies for cup quality analysis (e.g. flavour, acidity, body)(Tolessa et al. 2016). Chemical composition (e.g. caffeine, trigonelline and chlorogenic acid contents), on the other hand, can successfully be quantified using equipments like near infrared spectroscopy (NIRS) (Esteban-Díez et al. 2004; Santos et al. 2012).

Factors that determine production and quality of coffee

Coffee tree growth, productivity and bean qualities are likely influenced by coffee trees geographical origin and a variety of interacting environmental factors (Avelino et al. 2005; Bertrand et al. 2006 ; Geromel et al. 2008; Lara-Estrada and Vaast 2007; Villarreal et al. 2009). Shade level (Vaast et al. 2006), fertilizer rates (Clemente et al. 2015; Lara-Estrada and Vaast 2007) and genotypic differences (Avelino et al. 2005; Bertrand et al. 2006 ; Leroy et al. 2006) are also among the major factors affecting tree growth, productivity and coffee bean quality. Agronomical measures like pruning and fruit thinning (Bertrand et al. 2006 ; Cagliani et al. 2013; Cannell 1985; Läderach et al. 2011; Vaast et al. 2006) and soil physical and chemical characteristics (Behailu et al. 2008; Bosselmann et al. 2009) were also reported to influence yield, physical and chemical properties of coffee beans.

Shade and nitrogen fertilizer

In earlier production systems, coffee bushes were planted under shade canopy to simulate their natural habitat (Bosselmann et al. 2009; DaMatta 2004; Méndez et al. 2007). Coffee grown without shade potentially outyielded shade coffee (Beer et al. 1998; Bote and Struik 2011; DaMatta 2004). In Ethiopia, decreasing shade to increase coffee production caused losses of plant species diversity (Schmitt et al. 2010; Senbeta and Denich 2006). Model calculations showed that due to accelerated global climate change, 65 – 100 % suitable area for *C. arabica* production may be lost in the year 2080 (Davis et al. 2012). Habitat degradation, forest modification and clearance, especially for agriculture aggravate the loss even further (Ahrends et al. 2010)

Nitrogen (N) fertilization is essential for high yield of coffee, and N is the most required nutrient for vegetative growth and coffee bean development (Clemente et al. 2015). Poor availability of nitrogen is naturally limiting the performance of coffee production systems (e.g. Njoroge 1985). Given the need to increase production in the face of rising global demand, sustainable means of increasing production have become an increasing global concern (Perfecto et al. 1996). In this regard, the effects of shading and fertilizer use on coffee production and quality are of particular interest. However, quantitative understanding of the interactions between available nitrogen in the soil and level of radiation received by the coffee plant on coffee growth, bean production and bean quality is not available. In this context, the first hypothesis tested here was that gains in coffee productivity and quality can be made if management of shade and nutrients are based on more profound insight in dependency of nitrogen on the amount of available radiation.

Fruit load

Coffee plants tend to flower heavily resulting in the production of high fruit loads (DaMatta et al. 2008). A high fruit load demands proportionally more resources (assimilates and nutrients), which could otherwise be used for new vegetative development. For instance, production of 6 t of fresh berries ha^{-1} year^{-1} (yielding 1 t green coffee bean and 1.25 t dry pulp and parchment) exports 75 kg N, 11 kg P and 98 kg of K from coffee field (Van der Vossen 2005). Production of high fruit load without a concomitant balance in vegetative development leads to biennial bearing, means high fruit yields in ‘n’ year and low or no fruit yield in ‘n+1’ year (Guitton et al. 2011) and branch die-back, i.e., descending branch death (DaMatta et al. 2008). Biennial bearing results in severe loss of both yield and quality of coffee beans. Yet the dynamic of mechanisms causing these biennial patterns are still poorly understood and the means of adequately managing it are currently lacking. In apple (Meland 2009) and olive (Haouari et al. 2012) reduction of initial fruit load significantly reduced bienniality. This sets the need for crop load regulation to avoid coffee bean quality deterioration and premature aging of the crop. The extent to which crop load management may similarly increase berry yields and quality in coffee however is not well known. Taking this in to account, the second hypothesis tested was that thinning of fruits at their early stage modulates resource allocations among competing sinks, stabilize yields over years and improve bean quality.

Genotype and environment

Coffee bean productivity, physical characteristics and biochemical composition of the beans also appear to be influenced by genotype (Bertrand et al. 2006 ; Jha et al. 2014). Environmental conditions also play important role in yield stability and the development of attributes conferring good quality, indicating distinct coffee characteristics depending on where the coffee is grown. The maintenance over time of stable yield and specific qualities of a 'terroir' (Avelino et al. 2005; Boot 2005) requires insight in the degree to which coffee production is determined by the combination of climate and edaphic factors, prevalent at a site, on the one hand and the genotype on the other hand. However, it has not been established yet, to which extent genotype and the specific environments determine productivity and specific quality of Ethiopian coffee. Hence, the third hypothesis tested here was that maintaining coffees of specific flavours can be managed better if the interplays between genotype and environment are quantitatively described and their effects are correlated with change in bean quality and its specific attributes.

Development of integrated and effective management packages along the coffee production chain helps to ensure stable production of high yield and superior quality beans (Bertrand et al. 2008) and improves the competitiveness of the country in the world market. The current study is solution-focused study in the sense that it involves the generation and organization of knowledge on the main determinants of coffee growth, productivity and bean quality in order to contribute to such management packages. The most innovative aspect of the study is the quantitative description of effects of each growth, productivity and quality determinant. The results can be used in the development of decision-supporting tools that ensure sustainable coffee production system.

Research questions

To achieve this general goal and test the aforementioned hypotheses, the following research questions were addressed in the study.

1. How do coffee plants respond to different radiation levels and rates of nitrogen supply in terms of growth, morphology, biomass production and dry matter allocation (Chapter 2), uptake and distribution of nitrogen in the plant and first yield of coffee beans (Chapter 3)?
2. How do radiation and nitrogen treatments affect radiation interception and radiation use efficiency (Chapter 2); N recovery, physiological efficiency and photosynthetic properties of leaves (Chapter 3); bean physical and organoleptic properties (Chapter 5)?
3. How does fruit load regulation (by fruit thinning) reduce biennial bearing, enhance vegetative growth (Chapter 4) and improve coffee bean quality (Chapter 5)
4. To what extent do genotype and the growing environment affect coffee bean physical and

organoleptic attributes (Chapter 5)?

Outline of the thesis

This thesis is composed of six chapters including this general introduction (Chapter 1). Chapters 2 to 5 present the main results of the study. In Chapter 2, we report on the analysis of growth, i.e. plant height, numbers of pairs of plagiotropic branches and leaves, dry weights of plant compartments, biomass production and distribution, fractional light interception, LAI, and radiation use efficiency of 2.5 years old plants. Chapter 3 reports on uptake and distribution of nitrogen, photosynthetic properties and the first bean yield of plants of 3.5 years old. Chapter 4 examines the performance of coffee trees subjected to different fruit thinning treatments. Chapter 5 discusses how the available resources (radiation and nitrogen), tree management (fruit thinning), and genotype by environment interactions affect coffee quality. This specific chapter advances the hypothesis that coffee bean quality is promoted by factors and conditions that support non-limiting supply of resources for beans to grow and by a sufficiently long period of maturation. Finally, chapter 6 (General discussion) reports the main findings reported in the previous chapters and the overall contribution of the thesis. The chapter also contains recommendations and future research directions.

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Chapter 2

Analysis of coffee (*Coffea arabica* L.) performance in relation to radiation levels and rates of nitrogen supply I. Vegetative growth, production and distribution of biomass and radiation use efficiency

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Abstract

Intensively managed full-sun coffee *(Coffea arabica* L.), is potentially highly productive, but has shown disappointingly low yield performance, as adequate resource supplies (especially plant nutrition) are needed to sustain the productivity. In order to underpin rational radiation and nutrient management, the current study focussed on growth and development of 2.5 years old trees in relation to nitrogen supply in combination with several degrees of radiation. Three coffee varieties were grown under four levels of radiation (30 - 100% full sun) and four rates of nitrogen supply (0 - 88 g tree^{-1} y^{-1}), arranged in a randomized split-split plot design at Jimma University horticultural farm, Ethiopia, and their biomass increment (growth) and allocation, and crown characteristics were measured. Growth responded positively to both radiation and nitrogen supply, with positive interactions for several plant attributes (including number and length of branches, numbers of pairs of leaves per branch, radiation use efficiency). Plant height and area per leaflet declined with higher radiation level but the positive effect of larger N supply declined with increase in radiation. Branch length and leaf dry weight showed the most positive plasticity in response to higher radiation, but area per leaf showed a negative plasticity to higher radiation. Specific leaf area declined from 186.5 in shade (reducing sunlight to 30 %) to 108.8 cm^2 g^{-1} in full sun without effect of N. Positive effects of nitrogen on growth and biomass production were mediated through higher radiation-use efficiency, RUE, ranging from 0.23 to 0.45 g MJ^{-1} (PAR). Variables associated with dry matter partitioning were modestly responsive to either N or radiation. All these responses were consistent across the three varieties. The study enhanced the understanding of vegetative growth and biomass production of coffee trees and explored traits that underlie these patterns. The study also yielded essential information for developing coffee growth models, managing shade and nitrogen supply in both open sun and agroforestry systems.

Key words: growth analysis, plagiotropic branch, orthotropic stem, light extinction, radiation interception, specific leaf area, shade avoidance

Introduction

Coffee is the second most traded commodity after oil (Fenton et al. 2012), grown in 52 mostly low-income countries, and 70% of this coffee production comes from the species *Coffea arabica*. Given the need to increase production in the face of rising global demand, sustainable means of increasing production while limiting potentially negative ecological impacts, particularly on biodiversity, have become an increasing global concern (Perfecto et al. 1996). In this regard, the effects of shading and fertilizer use on coffee production are of particular interest.

C. arabica was originally classified as a shade obligate species, and traditionally cultivation is taking place under shaded conditions (Araujo et al. 2008; DaMatta et al. 2007). Using shade, especially in agroforestry systems, may enhance biodiversity, but low radiation availability may restrict production. Thus, to increase yield and income intensively managed full-sun coffee production systems were introduced in different parts of the world (Bruno et al. 2011; DaMatta et al. 2007; Harmand et al. 2007; Jaramillo-Botero et al. 2010). Yet, there have been disappointing experiences with full-sun cultivation and the consensus seems to be that under full-sun exposure adequate resources of nutrients (e.g. N) and water need to be provided to avoid problems such as reinforced biennial bearing and branch die back (Fahl et al. 1994; Siles et al. 2010) and reduced economic lifespan of the trees (Perfecto et al. 1996). However, the optimal combinations of radiation and nutrient supply are hard to define.

In order to underpin rational tree and system management, it is necessary to analyse the effects of radiation level (i.e. degree of shading) and nitrogen nutrition on tree performance. The qualitative responses of plants to different degrees of radiation and rates of nitrogen supply are relatively well known (Poorter 1999). Nevertheless, for coffee the magnitude of these responses, their potential interaction and traits underlying the patterns are poorly known, especially for young establishing plants. Even though a number of studies addressed aspects of effects of radiation and nitrogen supply (Harmand et al. 2007; Jaramillo-Botero et al. 2010; Montoyo et al. 1961), there is no comprehensive analysis of growth and development of young trees in relation to a range of rates of nitrogen supply in combination with several degrees of radiation. These two growth factors affect the rates of growth and development of young coffee plants in several ways. We adopt the following conceptual framework in order to structure the analysis of how these growth factors affect properties of the components of the coffee plant so as to ultimately explain the effects on growth and production. The daily growth rate per unit surface area occupied by a tree, ΔY (g m^{-2} d^{-1}) is modelled as a function of daily incident radiation, IR (MJ d^{-1}), the fraction of this radiation intercepted by the canopy, FI and radiation use efficiency, RUE (g MJ^{-1}) (Mariscal et al. 2000; Monteith and Moss 1977)

$$\Delta \mathrm{Y} = \mathrm{IR} * \mathrm{FI} * \mathrm{RUE} \qquad \text{(Eqn 1)}$$

IR is evidently directly related to shading, but also depends on the latitude and overall weather conditions in the coffee growing area. FI is primarily determined by the total leaf area per unit surface area, i.e. leaf area index, LAI, and by the extinction coefficient for light (k) (Monsi and Saeki 2005). LAI in turn, is determined by the number of leaves and area per leaflet, while k relates to architectural attributes such as leaf size and angle (Kitajima et al. 2005; Monsi and Saeki 2005); number, length and angle of branches (Kim et al. 2011; Kitajima et al. 2005). Allocation of structural dry matter to the component parts: root, trunk, leaves and branches indirectly affect leaf area per plant. Based on the functional equilibrium theory (Brouwer 1962; Lambers 1983), one would expect allocation to the shoot, particularly to leaves, to increase with larger nitrogen supply and larger degree of shading. Specific leaf area (cm^2 g^{-1}) captures the ‘conversion’ from dry matter allocated to leaves to leaf area and is a determinant of LAI and hence of *FI*.

The fraction of total plant dry weight allocated to leaves, LWR (g g^{-1}) is a determinant of relative growth rate of trees in early stages (Poorter and Remkes 1990). LWR measures the allocation of biomass to leaves versus other plant parts. Higher LWR means faster expansion of leaf area and light capture than in case of low LWR. Leaf area ratio, LAR (cm^2 g^{-1} or m^2 kg^{-1}) is the ratio between total leaf area per plant and total dry weight per plant. It also captures the relative investment in leaves, but includes SLA.

Radiation-use efficiency, RUE, is dependent on a number of factors including nitrogen rates (Colnenne et al. 1998) and factors inherent to plant species: leaf nitrogen, associated leaf photosynthetic capacity and photosynthetic nitrogen use efficiency, PNUE (Sinclair and Horie 1989), all of which can differ considerably between tropical tree species (e.g. Poorter and Bongers 2006; Selaya and Anten 2010).

This study analysed the performance of young coffee trees in relation to different combinations of radiation and nitrogen and addresses the following questions. (1) How do coffee plants respond to different radiation (shade) levels and rates of nitrogen supply in terms of growth, morphology (including numbers and sizes of leaves and branches, SLA), biomass production and dry matter allocation and leaf photosynthesis? (2) How do radiation and nitrogen treatments affect radiation interception and radiation use efficiency and first bean yields? (3) How do uptake and distribution of nitrogen as well as apparent nitrogen recovery respond to radiation and nitrogen? Three coffee varieties were used to justify generalization of the results to more than one genotype.

In this first paper, we report on the analysis of growth, i.e. plant height, numbers of pairs of plagiotropic branches (Arcila-Pulgarín et al. 2002; Cannell 1985) and leaves, dry weights of plant compartments, distribution of dry matter, fraction light interception, LAI, and RUE of 2.5 years old plants. The second paper on this study reports on (i) uptake and distribution of nitrogen, (ii) photosynthetic properties, and (iii) the first bean yield of plants of 3.5 years old.

Material and Methods

Description of the study site

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Horticulture farm, Jimma, Ethiopia (70^{o} 33'N , 360^{o}, 57' E and 1710 m a.s.l.). During the experimental period, the annual mean daily maximum and minimum temperatures of the site were 28.2 ^{o}C and 12.7 ^{o}C, respectively and the mean daily maximum and minimum relative humidity were 92.1 % and 49.4 %, respectively (data measured with data logger, Log Tag, HAXO-8, China, mounted in the study area). The area receives an annual rainfall of 1500-1800 mm.

Experimental design

Coffee (*Coffea arabica* L.) seedlings of variety 74-4, 74-40 and 74-110 were raised in shaded nurseries from May 2011 for fourteen months before being transplanted to the experimental site where treatments were applied. Healthy and uniformly grown seedlings were selected and transplanted on 20 July 2012 to polybags of 60 x 60 x 60 cm dimension, filled with a mixture of subsoil and sand (3:1 v/v), arranged under four different artificial shade levels using neutral nylon screens (Moplaco Packaging private limited company, Addis Ababa, Ethiopia) installed horizontally at a vertical height of 2.30 m above the ground.

The study was conducted using a randomized split-split plot design with four levels of radiation: 100%, i.e. open sun without any shade, 70, 50 and 30 per cent of full sunlight as a main plot factor abbreviated to R100, R70, R50 and R30, respectively. The main plots were split into three sub-plots to which the three coffee varieties were randomly assigned and each sub-plot was split into four sub-sub-plots to randomly accommodate four levels of nitrogen (0, 40, 64, and 88 g of N per plant per year, equivalent to 0, 150, 250 and 350 kg ha^{-1}), replicated three times. N treatments were abbreviated as N0, N40, N64 and N88, respectively. Each elementary plot consisted of six polybags (arranged in two rows of three) with one seedling per polybag. The spacing was in a grid of 1.6 m x 1.6 m between plants (i.e. a tree population density of 3906 ha^{-1}). Nitrogen, as urea, was applied in three split applications a year on 0, 116 and 236 DAT (days after transplanting) (i.e. 1-08-2012, 25-11-12 and 25-03-2013) in the first year and 374 and 487 DAT (10-08-2013 and 01-12-2013) in the second year, such that total applications corresponded with N treatments described above. Each tree also received 5 g P (63 kg ha^{-1} y^{-1} P) as Triple Super Phosphate (TSP) applied once a year, five days before N application on 26 July 2012 and 28 November 2013, following current recommendation (IAR, 1996). After application, both N and P fertilizers were thoroughly mixed into the soil to avoid losses from the soil surface. Based on the environmental conditions the seedlings were watered weekly, and hand-weeded as necessary.

Measurements

Non-destructively monitored growth traits

Observations on plant growth reported in this paper encompass the period from zero to 507 DAT, i.e. data are for trees between the age of 14 months (when they were transplanted) and 31 months. For growth analysis, two plants per plot were selected and on these plants two plagiotropic branches, i.e. second and third from the apex, were tagged on 1 November 2012. The third and fourth pairs of leaves counted from apex of the selected branches were also tagged as a reference position for evaluating change in leaf area and leaf dry weight. Data on plant height, number of pairs of plagiotropic branches, branch length and number of pairs of leaves per branch were all taken on monthly basis up to March 2013. At every sampling occasion, three to four leaves of similar positions as the non-destructively monitored reference leaves were detached and brought to the laboratory for determination of leaf area, leaf dry weight and leaf nitrogen. Gradually, branches selected in November 2012 had been overgrown by new branches. Therefore, from April 2013 onwards, the monitoring of branch growth was shifted to the top third/fourth branch pairs from the apex. In this selection, the number of plants was extended to four plants per plot. Branch length, number of leaf pairs per branch, leaf area per leaflet and dry weight per leaflet all were taken from these selected branches and leaves on these branches.

Light interception measurements

In the absence of local recordings, daily values of incident solar radiation of the site were taken from NASA (NASA 2015) providing coordinates of the experimental site. Incident daily radiations above the plants were calculated for each treatment by multiplying the daily solar radiation values with the transmissivity of the shade netting. Fraction light interception by each coffee tree was measured with a 0.8 m light rod below the crop and a reference sensor (Sunscan, Delta-T, UK) above the crop but underneath the shade netting. The light rod was positioned horizontally and light interception was measured, holding the rod from the edge to the centre of the plot, at 0.15 m intervals along the boundary of the plot so as to cover systematically the whole square area occupied by each coffee tree. Fraction of intercepted PAR was measured between 10:00 to 13:00 hr. on clear days (210, 275, 439, 472 and 507 DAT).

Destructive measurements

For growth analysis, fresh and dry weights of the coffee seedlings were determined at the time of transplanting. Subsequently, three coffee trees per treatment, one from each replicate, were harvested on 258, 350, 413 and 507 DAT (11 April 2013, 12 July 2013, 13 September 2013 and 16 December 2013, respectively). Samples were separated into stem, branches, leaves and roots. Plagiotropic branches are growing in pairs at angles of 180 ° from each phytomer on the orthotropic stem (Arcila-Pulgarín et al. 2002; Cannell 1985), hence, the number of pairs of branches is reported in this paper. Likewise, pairs of leaves are growing from each phytomer of plagiotropic branches. In order to make a clear distinction between individual leaves and pairs of leaves we use 'leaflet' to refer to the former. Roots were washed thoroughly over a 0.5 mm screen sieve. Leaf area was measured using a leaf area meter (AM- 200, Delta T devices Ltd., Cambridge, UK). Leaves were then oven dried at 70 ºC for 72 h., branches, stem and roots were dried at 105 ºC to a constant weight (Walinga et al. 1995). Dry weights of each plant part were measured with a precision digital balance (model CTG-6H, Citizen Scale plc., Parwanoo, India) with precision 0.01g. Most of the morphological variables presented in this paper are for coffee trees destructed on 507 DAT.

Calculations and statistical analysis

Fraction of biomass allocation within plants was computed in g per g of total biomass. LAI (m^2 m^{-2}) of each individual tree was calculated from leaf dry weight (g $tree^{-1}$), SLA (cm^2 g^{-1}) and surface area occupied per tree, SA (m^2). A conversion factor 10000 was used to convert cm^2 to m^2

$$LAI = \frac{LDW * SLA}{SA * 10000} \qquad \text{(Eqn 2)}$$

$$FI = 1 - e^{-k*LAI} \qquad \text{(Eqn 3)}$$

Light extinction coefficient (*k*), was estimated applying Eqn 3 (Monsi and Saeki 2005). *FI* was measured on 210, 275, 439, 472 and 507 DAT. However a daily *FI* value is needed to calculate the daily intercepted radiation from day 1 to 507 after transplanting. To that end, a cubic polynomial regression equation was fitted to *FI* values measured over time. Cumulative intercepted radiation, CIR, for the whole experimental period was obtained by adding the intercepted radiation of each day. Radiation use efficiency, RUE (g MJ^{-1}) (PAR basis) of the trees was then calculated by the ratio of increment in total dry mass between 0 and 507 DAT and cumulative intercepted radiation over the same time span:

$$\mathrm{RUE} = \frac{\mathrm{TDW_{507} - TDW_0}}{\mathrm{CIR}} \qquad \text{(Eqn 4)}$$

Where:

TDW_{507} is total dry weight (g $tree^{-1}$) at 507 DAT
TDW_0 is total dry weight (g $tree^{-1}$) at 0 DAT
CIR is cumulative intercepted radiation (MJ)

To analyse how underlying factors (nitrogen, radiation) contribute to the tree performance (differences in dry matter production), a growth response ratio (GRR) was calculated for each trait with Eqns 5a and b

$$\mathrm{GRR_x} = \frac{\mathrm{X_{N88}}}{\mathrm{X_{N0}}} \qquad \text{(Eqn 5a)}$$

$$\mathrm{GRR_x} = \frac{\mathrm{x_{R100}}}{\mathrm{x_{R30}}} \qquad \text{(Eqn 5b)}$$

Where:

X is variable (e.g. plant height) at a given nitrogen and / or radiation level
X_{N88} and X_{N0} are the mean values of variable X, respectively at N_{88} and N_0
R_{30} and R_{100} are the mean values of variable X, respectively at R_{30} and R_{100}

Most of the growth variables presented in this paper are data from the plants harvested at the end of the monitoring period at 507 DAT. Data were analysed with Statistical Analysis System (v. 9.2, SAS Institute Inc., Cary, NC USA,) software using the general linear model procedure for a split-split plot design. Significant differences between treatment means were separated using the Fisher's LSD-procedure at $p < 0.05$. *F* values of ANOVA results showed that the measured variables were strongly influenced by radiation, nitrogen and/or their interactions. Except where varietal differences were robust, for the subsequent analysis we pooled data across varieties and reduced data to radiation and nitrogen effects (Scheiner and Gurevitch 1993).

Results

Table 1. Three factor analysis of variance of growth variables measured for three coffee varieties, V, grown under different radiation levels, R(%), and rates of nitrogen supply, N (g tree^{-1}).

Factors	Pht	BrL	Brnr	Lfnr	LA	LDW	SLA	LAI	FI	RUE
R	**1641*****	**902*****	**207*****	16.1*	**198*****	**144*****	**216*****	**5.4***	**36.2*****	**3.3ns**
V	90***	339.7***	3.6ns	2.1ns	188***	115***	43.1***	ns	6.2*	ns
R x V	34.3*	6.6*	2.0ns	7.1**	27.4**	7.3*	24.6**	ns	5.4**	ns
N	**1382*****	**357*****	**180*****	**37*****	169***	**123*****	0.6ns	**50*****	**50*****	**54*****
R x N	**81.4*****	**9.9****	7.6**	1.6*	5.4*	1.4ns	2.4*	ns	2.1*	4.3*
V x N	39.4*	1.9ns	2.1ns	7.4***	7.3*	3.4*	**10.7****	ns	3.9**	ns
R xVxN	11.6*	6.7*	1.4ns	1.4ns	2.8*	1.6ns	4.2*	ns	3.1**	ns

Numbers in the table are *F*- ratios of data collected at 507 DAT. *F*-ratio of factors having the largest effect on the variables are given in bold. Growth variables are: plant height (Pht, cm); branch length (BrL, cm); number of pairs of branches (Brnr, #); number of pairs of leaves (Lfnr, #); leaflet area (LA, cm^2); leaf dry weight (LDW, g); specific leaf area (SLA, $cm^2\ g^{-1}$); LAI ($m^2\ m^2$);fraction of light intercepted (FI); and RUE ($g\ MJ^{-1}$). Ns = $p > 0.05$; $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$

Plant attributes at 507 DAT

Plant height declined with the level of radiation (Fig. 1a). Branch length (Fig. 1b), on the other hand, increased with the radiation level, though there were no significant differences ($p > 0.05$) between R70 and R100 treatments. Plant height as well as branch length responded positively to increased nitrogen supply, but both effects became relatively smaller at high radiation levels, especially for plant height (Figs. 1a and b), as reflected by the significant N by radiation interaction (Table 1).

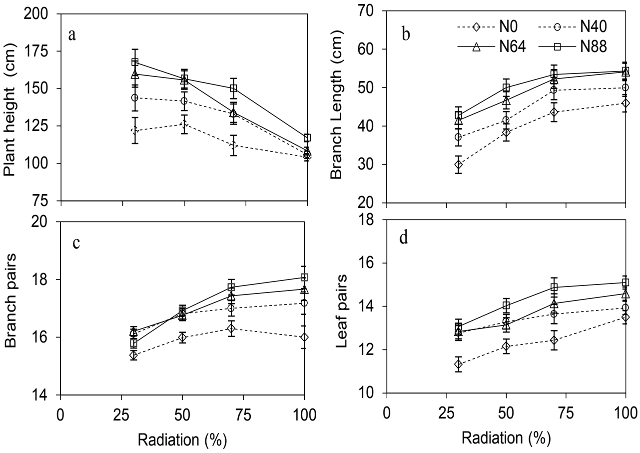

Fig. 1. (a) Plant height; (b) average branch length; (c) number of branch pairs per plant and (d) number of leaf pairs per branch as affected by radiation and nitrogen treatment levels. Data points are averages of nine values averaged across three varieties and three replicates; data from the last destructive measurement (507 DAT). Vertical bars indicate mean ± 1 SE. When not shown, SE was smaller than the symbol. For reference the values at DAT = 0 are provided: plant height 49.5 cm, branch length 15.6 cm, three pairs of branches per tree and four pairs of leaves per branch.

The number of pairs of branches per plant and the number of pairs of leaves per branch showed qualitatively similar patterns of positive response to radiation and nitrogen treatments as branch length. With more radiation and higher rates of nitrogen supply, both the number of pairs of branches (Fig 1c) and leaves (Fig. 1d) increased, the nitrogen effect being slightly larger at high than at low radiation. The varietal effect on numbers of branches and leaves was insignificant.

Area per leaflet was higher the lower the radiation level ($p < 0.05$, Fig. 2a); N supply tended to have a positive effect on leaf area but this effect declined with increasing radiation, and was non-significant for the comparison R70 and R100. In contrast to area per leaflet, dry weight per leaflet increased with radiation level, also for R100 compared to R70 (Fig. 2b). Nitrogen had a positive effect on dry weight per leaflet, but the differences between N64 and N88 were insignificant ($p > 0.05$). For leaf dry weight, there was no interaction between radiation and nitrogen supply (Table 1). Concerning varietal effect on leaf size and weight, it was noted that at full radiation and averaged across rates of nitrogen, area per leaflet was 40.0 cm^2 for variety 74-110, while it was 49.4 cm^2 for variety 74-40 and 45.7 cm^2 for 74-4. Area per leaflet of the latter two varieties did not significantly differ. Leaflet dry weight showed a similar pattern of responses to varieties as area per leaflet. At R30, averaged across nitrogen rates, leaflet dry weight of variety 74-110 (0.30 g) was significantly less than leaf dry weight of variety 74-4 (0.36 g) and 74-40 (0.35 g). The latter two varieties did not significantly differ from one another (*p* > 0.05).

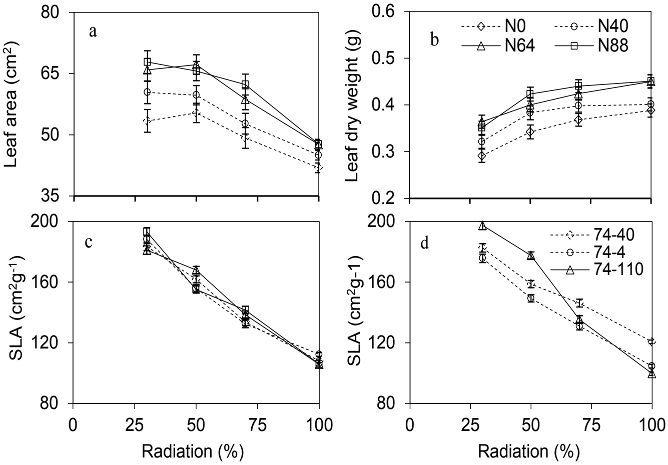

Fig. 2. (a) Area per leaflet; (b) Dry weight per leaflet; (c) SLA of coffee trees as affected by radiation and nitrogen treatments, (d) SLA of the three coffee varieties as a function of radiation. Data were taken from the last destructive measurements (507 DAT). In panels a – c each data point are averages of nine values averaged across varieties; in panel d, points are averages of 12 values averaged across nitrogen treatments. Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. For reference the values at DAT = 0 are provided: leaf area 40.5 cm^2, leaf dry matter 0.20 g.

Due to the opposite responses of area and dry weight of leaflets to irradiance, SLA increased strongly with increase in the level of shading (Table 1, Fig. 2c), with no significant differences between nitrogen treatments. Among varieties, variety 74-110 showed the lowest SLA at full radiation and the highest at R30, so this variety showed a stronger response to shade than the other two (Table 1, Fig. 2d).

LAI, light interception, dry matter accumulation and radiation use efficiency

Leaf area index, LAI, at 507 DAT increased with N supply, though differences between N64 and N88 were not significant. LAI tended to show a parabolic relationship with radiation level, LAI values at R50 and R70 tending to be larger than the values at R30 and R100 (Fig. 3a).

FI declined with radiation level: e.g., at 507 DAT, FI was 0.65 in R30 trees and 0.50 in R100 trees (Fig. 3b). Higher rates of N supply led to higher FI values, but this effect became smaller with increasing radiation level (Fig. 3b). Inserting data on FI and corresponding LAI values, measured at 507 DAT, in Eqn 2 yielded extinction coefficient, *k*, ranging from 0.38 at R100 to 0.51 at R30.

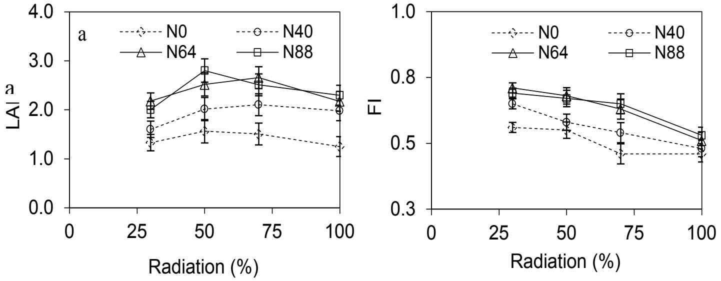

Fig. 3. (a) Leaf area index, LAI, per tree and (b) fraction of light intercepted, FI versus radiation for coffee trees grown under different radiation levels and nitrogen fertilizer rates. Data points are averages of nine values averaged over varieties taken at 507 DAT. Vertical bars indicate mean ± 1SE.

Table 2. Three factor analysis of variance of growth variables measured for three coffee varieties, V grown under different radiation levels, R(%) and rates of nitrogen supply, N (g tree^{-1}).

Factors	TDW	RSR	LWR	LAR	SLA
R	**1164*****	**85.7*****	**23.6****	**441.6****	**280.6*****
V	484.6***	**86.6*****	**33.7*****	**328.7*****	**31.9*****
R x V	7.8**	5.0*	7.7***	81.3***	17.4***
N	**1073*****	18.9***	12***	6.0*	ns
R x N	15.1***	ns	3.5***	2.7*	2.5*
V x N	85.7***	2.9*	14.4***	25.6***	7.0***
R x V x N	8.2**	3.8***	3.2***	3.93***	3.9***

Numbers in the table area *F*- ratios of data collected at 507 DAT. *F*-values of factors having the largest effect on the variables are given in bold. Growth variables are: Total dry weight (TDW, g tree^{-1}); root shoot ratio (RSR); leaf weight ratio (LWR, g g^{-1}); leaf area ratio (LAR, cm^{2} g$^{-1)}$; specific leaf area at tree level (SLA, cm^{2} g^{-1}) . Ns = $p > 0.05$**;** * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Destructive sampling at 507 DAT showed positive responses to radiation and nitrogen supply for increment in total biomass per plant (Table 2 and 3). The positive effect of nitrogen on biomass increased with radiation (i.e. significant positive interaction between radiation and nitrogen supply, Table 3). Biomass increment from N0 to N88 was 276 g for R30; for R100 the increment increased to 508 g. The differences between R70 and R100 at all rates of nitrogen rates, however, were not significant (p > 0.05).

Across N rates and varieties root to shoot ratio (R: S) increased with radiation level from 0.11 for R30 N88 to 0.19 for R100 N0. There was a tendency for R:S ratio to decrease with N supply (Table 3). LWR revealed an opposite response pattern as R:S; it decreased with higher radiation level but slightly increased with nitrogen supply. LAR and SLA showed remarkable increase in response to low radiation ($p < 0.001$, Table 3). Except at R50, nitrogen supply did not significantly affect LAR.

Table 3. Increment in total dry matter in the period 0-507 DAT (TDM g tree^{-1}), root to shoot ratio (RSR), leaf weight ratio (LWR, g g^{-1}), leaf area ratio (LAR, cm^{2} g^{-1}) and SLA (cm^{2} g^{-1}) of coffee trees as affected by different radiation levels (R%) and rates of nitrogen fertilizers (N g tree^{-1}).

Radiation	Nitrogen	TDW	RSR	LWR	LAR	SLA
30	0	431.9^{e}	0.130^{ef}	0.397^{b}	74.1^{a}	186.6^{a}
	40	573.6^{d}	0.114^{g}	0.401^{ab}	75.7^{a}	188.9^{a}
	64	714.5^{dc}	0.122^{gf}	0.386^{c}	70.2^{a}	182.1^{a}
	88	707.6^{dc}	0.120^{g}	0.403^{a}	75.59^{a}	188.3^{a}
50	0	629.7^{d}	0.141^{e}	0.381^{cd}	62.1^{ab}	162.9^{b}
	40	848.0^{c}	0.135^{ef}	0.377^{d}	58.8^{bc}	156.3^{bc}
	64	1016.3^{bc}	0.131^{f}	0.362^{e}	60.4^{b}	166.9^{b}
	88	1077.5^{bc}	0.129^{f}	0.397^{b}	61.3^{bc}	154.6^{bc}
70	0	738.3^{c}	0.164^{bc}	0.376^{d}	50.03^{c}	133.8^{c}
	40	985.9^{b}	0.158^{c}	0.367^{de}	48.7^{c}	132.6^{c}
	64	1194.2^{ab}	0.150^{d}	0.363^{e}	50.4^{c}	139.0^{c}
	88	1191.8^{ab}	0.148^{d}	0.395^{b}	56.3b^{c}	142.5^{c}
100	0	770.3^{c}	0.186^{a}	0.389^{bc}	42.1^{d}	108.1^{c}
	40	1069.1^{b}	0.170^{b}	0.331^{f}	37.7^{d}	114.0^{d}
	64	1208.8^{ab}	0.181^{a}	0.359^{e}	38.0^{d}	105.8^{d}
	88	1277.9^{a}	0.166^{bc}	0.368^{d}	39.0^{d}	107.3^{d}

Means are averages of nine values averaged across varieties with three replicates. Numbers followed by the same letter in the same column are not significantly different according to LSD test (5%). For reference the values at DAT = 0 are provided: TDW 38.81 g tree^{-}1, RSR 0.37, LWR 0.32 (g g^{-1}) and LAR 50.10 cm^{2} g^{-1}.

Dry matter distribution

In the period from zero to 507 DAT, there were changes in the distribution of dry matter over component plant parts. The fraction of plant dry matter allocated to roots declined from 0.24 to 0.13 (Fig. 4a). Within shoots, the fraction allocated to stem declined from 0.45 at 0 DAT to 0.35 at 507 DAT (Fig. 4b); in leaves, however, the fraction increased from 0.36 to 0.44.

At 507 DAT, there were significant interactions between the effects of radiation and nitrogen supply on biomass partitioning to shoots, roots and within shoots (detailed data not shown). Fraction of biomass partitioned to shoot in general and to stem in particular increased with increasing both shading and N supply. Shading, on the other hand, decreased the fraction partitioned to leaves from 0.47 in R100 to 0.40 in R30. The fraction of biomass partitioning to branches was on average 0.20 and was conservative across radiation and nitrogen treatments. There were no differences in biomass partitioning between the three coffee varieties neither as a main effect nor as an interaction with radiation and with nitrogen supply.

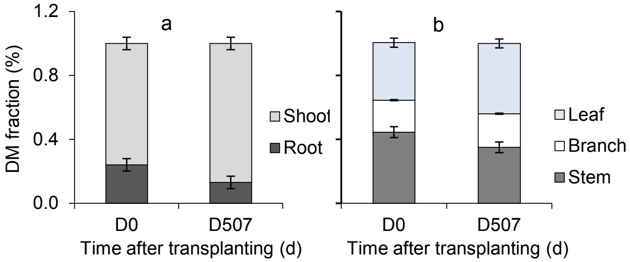

Fig. 4. Average fractions (a) of roots and shoots in total biomass and (b) distribution of shoot biomass over leaves, branches and stem of coffee trees grown under different levels of radiation and nitrogen. D0 indicate initial data taken from the three coffee varieties in three replicates. D507 indicates data taken from the destructive measurements at 507 DAT, averaged across varieties, radiation and nitrogen (48 data points). Vertical bars are mean ± 1SE.

Across treatments, over the period 0 - 507 DAT, radiation use efficiency, RUE, ranged from 0.23 to 0.45 g MJ^{-1}. RUE increased with increase in shading and with higher N rate (Fig. 5). The interaction between radiation and nitrogen on RUE was also significant ($p < 0.05$): in R30 increase in N rate from 0 to 88 g $tree^{-1}$ enhanced RUE from 0.31 to 0.43 g MJ^{-1}; the corresponding numbers in R100 were 0.23 and 0.43 g MJ^{-1}, i.e. larger positive effect of N supply in full sun than in shade. In N88 and N64 treatments RUE was the same irrespective of radiation level, whereas in N0 and in N40 treatments RUE tended to decline with increasing radiation (e.g. for N0 RUE dropped from 0.31g MJ^{-1} for R30 to 0.23 g MJ^{-1} in R100).

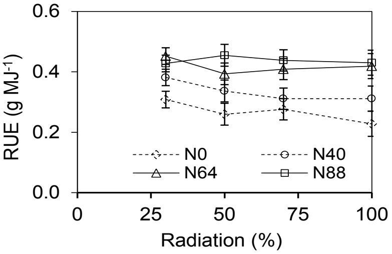

Fig. 5. Radiation use efficiency, RUE, of coffee trees grown under different growth radiation and nitrogen fertilizer rates. Data points are averages of nine values averaged over varieties taken at 507 DAT. Vertical bars indicate mean ± 1SE.

Discussion

In this study, we addressed the question to what extent radiation level and nitrogen supply influence performance of vegetative coffee trees, expressed here as biomass, and aimed to explore trait responses that underlie these patterns. We found positive responses of tree growth and biomass production to radiation and nitrogen: at 507 DAT, R100 plants had produced 1.64 - 1.7 times as much biomass as R30 plants (averaged over N treatments) (Table 4); likewise, N88 plants had produced 1.74 to 1.9 as much biomass as N0 plants (averaged across radiation levels). There was interaction between radiation and nitrogen, with the stimulatory effects of nitrogen on biomass being larger the higher the level of radiation (Table 2, Table 3). Between extreme treatments combinations (R30N0 vs R100 N88) a difference of a factor of about three was found in biomass at 507 DAT (Table 3).

The positive effects on biomass accumulation of higher radiation and larger rate of N supply were achieved by different mechanisms. For instance, higher radiation level resulted in smaller but much thicker leaves, while increased N supply gave larger leaves but did not alter SLA. In order to systematically show these different modes of action Table 4 summarizes GRRs. Arbitrarily broken down into classes of 0.2 unit points. The larger the difference with unity (positive or negative) the larger the plasticity of the trait in question to radiation and N supply. Traits with GRR values in the range of 0.8 to 1.2 can be considered comparatively indifferent to experimental factor in question, while traits with GRR values ranging from 1.2 to 1.4 are intermediately responsive and GRRs > 1.4 are considered very responsive. For nitrogen the ranking of the variables in the categories 1 - 1.2 and 1.2 - 1.4 indicates that the plasticity of organ properties such as length, weight and area is larger than the plasticity in organ number.

For radiation lower values for area per leaflet, plant height, SLA and LAR in high radiation environments than in low radiation environments seem to counteract better performance observed in high radiation. So does the high GRR for the root to shoot ratio for radiation, which also falls in the category of largest plasticity (GRR 1.6 - 1.8; Table 4). Branch length and leaf dry weight showed the most positive plasticity in response to both radiation and N supply. For nitrogen the most sensitive trait was RUE; it increased from 1.39 for N0 to 1.8 for N88 (Table 4) however, this effect was much stronger at high (~86%) than at low radiation (~40%) (Fig. 5).

Table 4. Growth response ratios, GRR (Eqns 5a and 5b) showing maximum effects of nitrogen and radiation levels on tree attributes.

GRR	Nitrogen	Radiation
1.4 – 1.8	RUE	RSR Branch length
1.2 – 1.4	Plant height Branch length Leaf Dry weight Leaf area	Leaf dry weight
1.0 – 1.2	Leaf number Branch number LWR	Leaf number LWR Branch number
0.8 – 1.0	RSR LAR SLA	RUE
0.6 -0.8	-	Leaf area Plant height SLA LAR

For simplification GRR values were averaged respectively across radiation and nitrogen rates. In general, GRR >1 indicate a strong positive response (large plasticity) of the trait in question for changes of the experimental factor in question. GRR values for plant biomass as a function of radiation and nitrogen levels are, respectively 1.62 – 1.71 and 1.74 – 1.86. Interactions are not visible in Table 4 but were generally maximally equivalent to 0.2 unit points of GRR.

Reduction in area per leaf and concomitant decline in SLA with more radiation are among the most conspicuous results of this study. More radiation resulted in a higher number of leaves; the opposing reactions of leaf size and leaf number more or less cancelled each other out such that LAI was not much affected by radiation level. In some other studies e.g. Campanha et al. (2004) similar effects of radiation on stimulating leaf number and reducing area of individuals leaves have also been observed. Ecological benefits have been attributed to smaller and thicker leaves at high radiation levels: smaller and thicker would help to maximize water-use efficiency (Parkhurst and Loucks 1972) partly because the boundary resistance increases with leaf size such that smaller leaves in high radiation conditions are better capable of maintaining favourable temperature. Smaller leaves may also allow more light to pass to lower canopy layers, which is beneficial in high radiation environment, where top leaves receive light in excess, saturating leaf photosynthesis.

Low radiation stimulated stem elongation but inhibited branch growth. Greater growth in stem height (Morais et al. 2006) and reduced branch growth (Campanha et al. 2004) have also been reported for coffee trees grown under shade compared to trees exposed to full sun.

This stimulation of stem elongation is typically considered as part of the ‘shade avoidance syndrome’ (Smith and Whitelam 1997), a suite of responses that is induced by neighbour plant shading. The response enables plants to overtop neighbour plants and thus prevent having to grow in the shade. However, it comes at costs as we observed lower LWR the lower the radiation level (Table 3). In habitats where companion plants always grow much taller than the species under consideration, such as would be the case in a coffee agroforestry system, shade avoidance responses are not beneficial as the investment in stem elongation does not confer advantages in light capture (Dudley and Schmitt 1996). In this study, the shade avoidance measured by differences in plant height (Fig. 1a) was stronger at high N supply than at low N supply. At low N supply, the resources may be limiting to fully express shade avoidance reactions. However, these results suggest the fertilizer application in shade grown coffee may have undesirable effect of strengthening shade avoidance and associated stem elongations.

To the best of our knowledge, this is the first paper to report values for radiation use efficiency in coffee. Nitrogen supply positively affected RUE as observed in wheat (Caviglia and Sadras 2001; Latiri-Souki et al. 1998), maize and sorghum (Muchow and Sinclair 1994), This positive effect of nitrogen on RUE was the major pathway through which nitrogen stimulated tree performance the higher the radiation level. In agreement with the relatively low annual carbon accumulation rate of coffee and similar species (Kattge et al. 2009), the values of RUE were low (0.23 – 0.45 g MJ^{-1}, Fig. 5) compared to values often larger than 2 g MJ^{-1}(PAR) for healthy C_3 crop plants like cereals (Monteith and Moss 1977; Sinclair and Horie 1989). But, the values are comparable with 0.5 g MJ^{-1} (PAR) reported for young *Eucalyptus* trees (Linder 1985) and also to values reported for mid-to late successional tropical trees (Selaya and Anten 2010). These relatively low RUE values are associated with the low photosynthetic capacity of the coffee trees of 4 - 11 µmol m^{-2} s^{-1} CO_2 (Araujo et al. 2008; DaMatta 2004; Martins et al. 2014).

Vos and van der Putten (1998) and Anten et al. (1995) suggested that there are two extreme strategies of plant response to nitrogen limitations. In strategy I, plants maintain their leaf size, maximizing light interception to the detriment of leaf nitrogen concentration, photosynthetic capacity and RUE. In strategy II, plants adapt their leaf size to maintain high nitrogen content per unit leaf area, Na, photosynthetic capacity and RUE to the detriment of light interception per plant. According to the GRRs (Table 4), coffee shows features of both strategies. The high GRR for RUE hints at nitrogen dilution when N becomes limiting as in strategy (I), but the GRR for leaf area (slightly above 1.2) is a feature of strategy (II).

As far as we know, there are no previous studies provided such physiological information explaining biomass production in coffee trees and the strategies that the trees are using to maximize the production. This study brought new insight on how coffee trees in high radiation environment maximized biomass production. In addition, this study is the first of its kind reporting light extinction coefficient, k for coffee trees. We estimated light extinction coefficient, $k = 0.44$ for coffee trees grown with non-limiting resources in a grid of 1.6 by 1.6 m. Since RUE and k are key elements in many crop growth models, the values presented here for both RUE and k can be used for bridging the gap that limited coffee growth model development.

Compared to many other traits, variables associated with partitioning such as RSR and LAR for nitrogen and LWR for both nitrogen and radiation were modestly responsive (Table 4). For radiation, however, the variables RSR and LAR were the most responsive ones: radiation positively affected RSR as was also observed in oak plant communities (van Hees and Clerkx 2003) but negatively affected LAR with larger effect the lower the N supply (Table 3).

Conclusion

This study unravelled the interactive and individual effects of radiation and nitrogen supply on vegetative growth and biomass production of coffee trees. Interactive effects of radiation and nitrogen were stronger at organ levels than at plant level (Tables 1 and 2). Positive interactions were shown for length (Fig. 1b, Table 1) and the number of branches, (Fig. 1c and Table 1) the number of leaves per branch (Fig. 1d and Table 1) and radiation use efficiency (Table 1and Fig. 5). Negative interactions were seen for plant height (Fig. 1a) and area per leaf (Fig. 2a and Table 1) and the fraction of intercepted radiation. Radiation stimulated root biomass, root to shoot ratio, leaf dry weight but reduced area per leaflet, SLA and leaf area ratio. Larger N supply stimulated stem extension, area per leaflet, leaf dry weight and RUE. Increased RUE with larger supply of N is a major driver of improved tree performance, followed by larger dimensions of branches and leaves that enhanced light interception. Phenotypic variability due to varietal differences was small indicating that varieties had similar eco-physiological responses to radiation and N supply. The study therefore provided information for developing a coffee growth model and managing shade and nitrogen supply in both open sun and agroforestry systems.

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Chapter 3

Analysis of coffee performance (*Coffea arabica* L.) in relation to radiation level and rate of nitrogen supply II. Uptake and distribution of nitrogen, leaf photosynthesis and first bean yields

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Abstract

Natural supply of nitrogen is often limiting coffee production. From the viewpoints of growth and biomass production, adequate nitrogen supply is important. Growing coffee under full sunlight not only enhances potential yields but also increases demands for nitrogen fertilizer, the extent of which is ill quantified. This paper provides a comprehensive analysis of N uptake and distribution, biomass production, photosynthetic characteristics of 2.5 years old trees and first bean yields of 3.5 years old coffee trees in response to four radiation treatments (30 % to 100% of full sun), factorially combined with four rates of nitrogen supply (0 to 88 g tree^{-1} y^{-1}). The experiment was arranged in a randomized split-split plot design and was conducted at Jimma University horticultural farm, Ethiopia, using three coffee varieties. With larger N application and higher level of radiation, more N was utilized and more biomass and yield were produced. The nitrogen recovery ranged from 7 to 17 % and declined with larger N supply and increased with radiation level. Coffee trees provided with larger amount of N had higher amounts of N per unit leaf area, light-saturated rate of leaf photosynthesis and first bean yield compared to trees grown in low N supply and limited radiation. The relation between biomass and plant N content was conservative across coffee varieties and can be used to estimate N content from biomass or calculate required uptake to produce a given amount of biomass. The relation can also be used in the development of process based quantitative coffee trees growth models. Achieving synchronies between N supply and coffee trees demand without excess or deficiency requires further investigation of options to improve the low nitrogen recovery.

Key words: N uptake, apparent nitrogen recovery, physiological efficiency, biomass, radiation, partitioning

Introduction

Poor availability of nitrogen is naturally limiting the performance of plant production systems, including coffee (e.g. Njoroge, 1985). Much of the research on effects of nitrogen in coffee is undertaken to underpin recommendations for nitrogen fertilization, considering economic and environmental aspects. Often such dose-response studies pertain to mature trees (Endris et al. 2008; Njoroge 1985). In coffee, it has been estimated that on average only 30 - 40 % of the applied N is taken up by the crop (Salas et al. 2002). The uptake percentage also decreases as the N-input increases (Bruno et al. 2014; Cannavo et al. 2013; Fenilli et al. 2007; Nunes et al. 1993). Low recovery of N in coffee could mean that appreciable fractions of supplied N are sequestered by competitors to coffee present in the system; these include soil microbes, weeds and shade vegetation if present (Tully et al. 2012). Especially during episodes when precipitation exceeds evapotranspiration large emissions of nitrogen to the environment can occur (denitrification and leaching (Bruno et al. 2014)). Dose response data are to a large degree site specific. Factors like natural soil fertility, as reflected in tree performance at zero nutrient input, and tree population density, as well as the degree of shade, affect the shape of the response curve. Regarding the latter, growing coffee at full radiation may enhance yields but also increases demands for nitrogen fertilizer, thus much low-input coffee is grown in shade.

For coffee, the relations between N demand and radiation level (degree of shade) are ill quantified. Also there is limited information on the underlying growth and developmental processes that in concert determine the plant's response in term of biomass and/or yield to N supply (Bruno et al. 2011; Fahl et al. 1994), particularly regarding the initial growth and the interaction with shade. It is the objective of this study to provide a comprehensive analysis of the initial growth, development, photosynthetic characteristics and first bean yields of young coffee trees in relation to factorially combined levels of nitrogen supply and radiation (i.e. degree of shade). Chapter 2 reported plant development, dry matter partitioning, radiation interception and radiation use efficiency. The objectives of this second paper are the analysis of the effects of radiation and nitrogen nutrition on: (i) uptake and distribution of nitrogen in the plant and dry matter production of 2.5 year old vegetatively growing plants; (ii) N recovery and physiological efficiency, (iii) photosynthetic properties of leaves as dependent on treatment, nitrogen per unit leaf area (N_a) and specific leaf area (SLA) and (iv) the response of the first bean yields of 3.5 years old plants to nitrogen and radiation treatments.

Material and Methods

Description of the study site

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine Horticulture farm, Jimma, Ethiopia (70^{o} 33'N , 360^{o}, 57' E, 1710 m a.s.l.). During the experimental period, the annual mean maximum and minimum temperature of the site were 28.2 ^{o}C and 12.7 ^{o}C, respectively and the mean maximum and minimum relative humidity were 92.1 % and 49.4 %, respectively (data, measured with data logger, Log Tag, HAXO-8, China, mounted in the study area). The area receives an annual rainfall of 1500-1800 mm.

Experimental design

Coffee (*Coffea arabica* L.) seedlings of varieties 74-4, 74-40 and 74-110 were raised in shaded nurseries from May 2011 for fourteen months before being transplanted to the experimental site where treatments were applied. Healthy and uniformly grown seedlings were selected and transplanted on 20-07-2012 to large polybags of 60 x 60 x 60 cm dimension filled with subsoil **:** sand (3 **:** 1 v/v) arranged under four different artificial shade levels using neutral nylon screens (Moplaco Packaging private limited company, Addis Ababa, Ethiopia) installed horizontally at a vertical height of 2.30 meter above the ground.

The study was conducted using a randomized split-split plot design with four levels of radiation: 100 % i.e. open sun without any shade, 70, 50 and 30 per cent of full sunlight as a main plot factor; these treatments will be abbreviated to R100, R70, R50 and R30. The main plots were split into three sub-plots to which the three coffee varieties were randomly assigned. Each sub-plot was split into four sub-sub-plots to randomly accommodate four levels of nitrogen (0, 40, 64, and 88 g of N per tree per year, equivalent to 0, 150, 250 and 350 kg ha^{-1}), replicated three times. N treatments will be abbreviated to N0, N40, N64 and N88. Each elementary plot consisted of six polybags (arranged in two rows of three) with one seedling per polybag. The spacing was in a grid of 1.6 m x 1.6 m between plants (i.e. a tree population density of 3906 ha^{-1}). Nitrogen, as urea, was applied in three split applications a year on 0, 116 and 236 days after transplanting (DAT) (1-08-2012, 25-11-12 and 25-03-2013) in the first year and 374 and 487 DAT (10-08-2013 and 01-12-2013) in the second year and two times 681 and 798 (12-06-2013 and 07-10-2013) in the third year, such that the totals equalled the levels listed above for N0 – N88. Each tree received 5 g P (63 kg ha^{-1} y^{-1} P) as

Triple Super Phosphate (TSP) applied once a year five days before N application on 26-07-2012 and 28-11-2013, following current recommendation (IAR 1996). After application, both N and P fertilizers were thoroughly mixed into soil to avoid losses from soil surface. Based on the environmental conditions the seedlings were watered regularly, and hand-weeded as necessary.

Measurements

Destructive measurements

Three coffee trees per treatment were destructively harvested on 258, 350, 413 and 507 DAT (11-4-2013, 12–07-2013, 13-09-2013 and 16-12-2013, respectively). Samples were then separated into stem, branches, leaves and roots. Roots were washed thoroughly over a 0.5 mm screen sieve. After drying each plant part to a constant weight, their dry weights were measured with precision digital balance (model CTG-6H, Citizen scale plc., Parwanoo, India) with precision 0.01 g and total N concentration was determined by Kjeldahl method after subjecting the dried samples to digestion by H_2SO_4-H_2O_2. The results presented in this paper are from destructive measurements collected at 507 DAT.

Leaf gas exchange variables

For the determination of light response curves (referred to by ‘Series 1’ in this paper), leaf net photosynthetic measurements were done at light levels of 0,100, 200, 300, 600, 1000 and 1500 µmol $m^{-2}s^{-1}$, provided from an external source, used with an infrared gas analyser LCpro+ (ADC BioScientific, Hoddesdon, U.K.); recordings were made at ambient temperature and CO_2 concentration on clear days. The temperature in the leaf chamber ranged between 25 and 30 °C. Measurements were done from 9:00 to 13:00 on six days between 423 to 447 DAT (23 and 26 September; 5 and 7 October; 16 and 17 October 2013) on fully developed leaves of third/fourth branch pairs counted from the apex of trees in two radiation levels (R100 and R50), two nitrogen rates (N0 and N88) and two varieties (74-40 and 74-110). After each measurement, leaves were detached and then taken to the laboratory for leaf area, leaf dry weight and total nitrogen contents determination.

To further explore effects of all treatments of radiation and nitrogen fertilizer rates on gas exchange variables, the net rate of carbon assimilation (A_{600}, µ mol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol CO_2 m^{-2} s^{-1}), transpiration rate (E, mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i, mmol mol^{-1}) were measured only at saturating incident radiation of

600 µmol m^{-2} s^{-1} between May 16 to 20, 2014 (393 - 397 DAT) (these measurements are referred to by 'Series 2' in this paper). At each time of data collection, measurements were done on fully developed third pairs of leaves, counted from the apex of the fourth pair of plagiotropic branches, counted from the plant apex. with the LCpro+ infrared gas analyser. Series of measurements were taken from 10:00 - 13:00 h. after 15 to 20 minute adaptation time to reach a steady-state condition. During measurement chamber temperature was 25 ± 3°C, while CO_2 (360 - 380 µ mol mol^{-1}) and relative humidity (70 - 80%) were kept at ambient.

Calculations and statistical analysis

At each destructive measurement, total biomass per tree was calculated as a sum of the dry weights of all the components. Total nitrogen per tree was also determined by adding total nitrogen content in each compartment. Apparent nitrogen recovery, ANR (fraction), was calculated by dividing the difference between the amount of N taken up by fertilized coffee (N_{uf}; g $tree^{-1}$) and unfertilized coffee (N_{u0}, g $tree^{-1}$) by the amount of fertilizer applied to fertilized coffee (N_{app}, g $tree^{-1}$) (Eqn 1):

$$\mathrm{ANR} = \frac{\mathrm{N_{uf}} - \mathrm{N_{uo}}}{\mathrm{N_{app}}} \qquad \text{(Eqn 1)}$$

N_{app} being the cumulative amount of N applied over the period 0 to 374 DAT. In Eq 1 ANR is expressed as a fraction, but percentages are also used to quantify ANR.

Physiological efficiency, PE (g g^{-1}), was calculated using Eqn 2.

$$\mathrm{PE} = \frac{\mathrm{DM_f} - \mathrm{DM_0}}{\mathrm{N_{uf}} - \mathrm{N_{uo}}} \qquad \text{(Eqn 2)}$$

where: DM_f and DM_0 (g) are total dry matter produced, respectively by fertilized and unfertilized coffees trees; N_{uf} and N_{u0} (g) are nitrogen taken up, respectively by fertilized and unfertilized coffee trees (Vos 2009).

Light response curves were fitted according to Thornley (1976) using a non-rectangular hyperbola model for photosynthesis (Eqn. 4) using SAS statistical software:

$$\mathrm{A_{net}} = \frac{\emptyset\mathrm{I} + \mathrm{A_{max}} - \sqrt{\{(\emptyset\mathrm{I} + \mathrm{A_{max}})^2 - 4\emptyset\mathrm{I}\theta\mathrm{A_{max}}}}{2\theta} - \mathrm{R_d} \qquad \text{(Eqn 3)}$$

where A_{net} = is the net photosynthetic rate (µmol $m^{-2}s^{-1}$)

I = incident photon flux density (µmol $m^{-2}s^{-1}$ (PAR))

$A_{\max}$ = the light-saturated rate of gross photosynthesis (μ molm^{-2}s^{-1})

$\emptyset$ = the initial slope of the curve (mol mol^{-1})

R_d = rate of dark respiration (μmol m^{-2}s^{-1})

θ = curvature (convexity) of the light-photosynthesis response curve (dimensionless)

First yield of berries was harvested from 10 to 20 December 2014 (872 to 883 DAT). Fresh weight of harvested berries per tree was determined and converted to dry clean beans (moisture content ca. 11%) with a conversion factor: 5.5 kg fresh berries = 1 kg clean, dry beans (Arcila-Pulgarín et al. 2002). Data were analysed with Statistical Analysis System (v. 9.2, SAS Institute Inc., Cary, NC USA,) software using the general linear model procedure for a split-split plot design with main plot (radiation), sub plots (varieties) and sub sub plot (nitrogen). Significant differences between treatments, means were separated using the Fisher's LSD-procedure at $p < 0.05$. Linear and quadratic regressions were also used to test relationships between variables.

Results

Analysis of the nitrogen response at 507 DAT

The increment in the amount of dry matter produced per tree over the duration of the monitoring period of 507 days increased with the cumulative amount of N applied in that period (Fig. 1a). Up to a cumulative N application of 79 g tree^{-1} (i.e. for data from the N0, N40 and N64 treatments), there was fairly a linear response of dry matter production to N applied. When more N was applied than 79 g tree^{-1} (in treatment N88), the response levelled off. The slopes of the lines up to 79 g tree^{-1} (Fig. 1a) were 3.49, 4.30, 5.82 and 5.59 dry matter produced per gram of applied nitrogen in the treatments R30, R50, R70 and R100, respectively. Regression analysis showed slopes were significantly different from each other ($p < 0.05$), except for R70 and R100 ($p > 0.05$). The amount of dry matter produced at zero N applied, i.e. the intercepts in Fig 1a, depended on the radiation level; this amount was 432 g tree^{-1} in R30 and increased to 771 g tree^{-1} in R100, indicating that under severe shade radiation was more limiting than availability of nitrogen in N0.

N uptake at zero N supply depended on radiation level. At R30, the uptake was 5.13 g tree^{-1} and was significantly lower than the uptake at R50 through R100 (8.36 g tree^{-1} on average). In all radiation treatments N uptake increased with larger amount of N applied, the increments were larger the higher the radiation level. Beyond 79 g N applied, however, the marginal gain in N uptake per unit N applied was much less than before that point, indicating strong decline in recovery. The relation between N supply and dry matter gain per tree over the period 0 - 507 DAT as a function of incremental N uptake over that period (Fig. 1c) was slightly curvilinear, indicating faster increase in N content than in dry matter weight. For a given N uptake, dry matter production tended to be slightly higher at high than at low radiation; for nitrogen, the reverse was true: slightly more dry matter per unit uptake in low N than in high N conditions (Fig. 1d). Across treatments incremental dry matter production, Y, was related to incremental N uptake, x by the regression (n = 48, $R^2 = 0.85$; numbers in the bracket represent SE of the coefficients):

$$y = 315.7\ (41.2) + \ 37.9(2.63)x - 0.016(0.001)x^2 \qquad \text{(Eqn 4)}$$

The apparent nitrogen recovery, ANR, increased with radiation level but declined with increasing cumulative N supply per tree. Values were generally low, ranging from 7 % for R30 to maximally 17 % for R100. Across all data, the physiological efficiency, PE, i.e. the

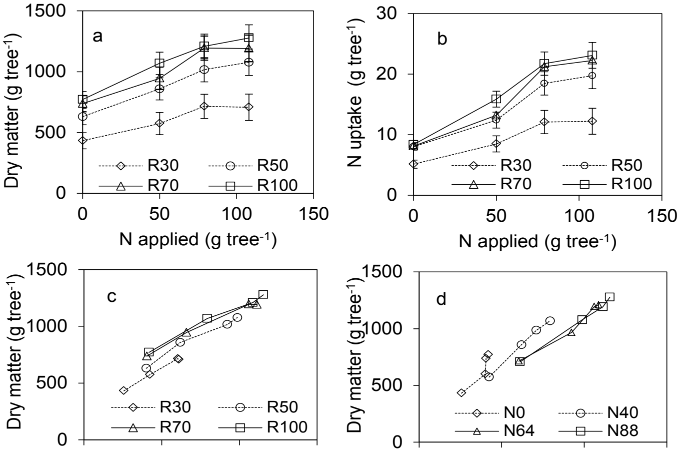

Fig. 1. (a) Incremental dry matter productions and (b) incremental N uptake in the period 0-507 DAT by coffee trees per unit of nitrogen applied; (c) dry matter production per unit of nitrogen taken up as a function of (c) radiation and (d) nitrogen rates over the period of 0 - 507 days. Data points are averages of nine values averaged across three varieties and three replicates taken from the last destructive measurement (507 DAT). Vertical bars indicate ± 1 SE and when not shown are less than the symbols. For references: at 0 DAT N content was 1. 85 g tree^{-1} and biomass was 38.81 g tree^{-1}.

amount of extra dry matter produced per unit of N taken up (between 0 and 507 DAT) by fertilized coffee trees relative to trees in N0, showed a slightly decline with increase in N uptake. The values ranged from 34 for N88 to 52 for N0.

Treatment effects on the average nitrogen concentration in plant dry matter (Figs. 2a and b) explain the shape of the curve in Figs. 1c and d. Across treatments, nitrogen concentrations varied from 12 to 19.8 mg g^{-1} in the dry matter of the whole plant (Fig. 2a) and from 1.33 to 2.78 g m^{-2} (N_a) in leaves (Fig. 2b). N concentrations in the whole plant (mg g^{-1}) and leaf (g cm^{-2}) increased with the rate of N supply though there was no difference between N64 and N88 ($p > 0.05$). This implies that the concentrations for N64 and N88 in Figs. 2a and b represent the maxima for the given plant masses and leaf area. In N0 and N40, N concentration in the dry matter of the whole plant declined with increase in radiation level. In N64 and N88, however, the concentration was slightly higher in R50 and R70 than in R30 and R100. In leaves, N_a increased with increasing radiation levels: in R30 N_a increased from 1.33 in N0 to 1.71 g m^{-2} in N88. In R100, the corresponding increase was from 1.79 to 2.78 g $m^{-2.}$ (Fig. 2b), i.e. there was a positive interaction between N and radiation.

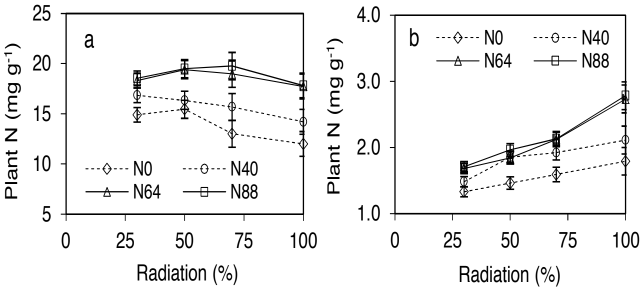

Fig. 2. Nitrogen concentration (a) per unit dry matter of coffee trees and (b) per unit leaf area of leaf, N_a, as affected by growth radiation and nitrogen fertilizer rates. Data points are averages of nine values averaged across varieties taken from the last destructive measurement (507 DAT). Vertical bars indicate mean ± 1 SE.

Nitrogen concentration in the dry matter of each organ varied across radiation and nitrogen treatments (Figs. 3a and b). In leaves, the value ranged from 1.93 mg g^{-1} for R100 N0 to 3.38 mg g^{-1} for R30 N88. The corresponding ranges in branch, stem and roots were, respectively 0.6 to 1.31, 0.36 to 1.04 and 1.48 to 2.43 mg g^{-1}

Nitrogen distribution over component plant parts

In the period from zero to 507 DAT, there were changes in nitrogen distribution over

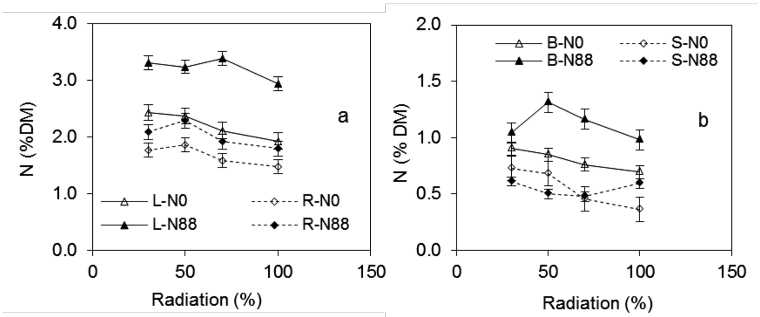

Fig. 3. Nitrogen concentration per unit dry matter of (a) leaf (L) and root (R); (b) branch (B) and stem (S) of coffee trees grown under different growth radiation and nitrogen nutrition. Data points are averages of nine values averaged across varieties taken from the last destructive measurement (507 DAT). Vertical bars indicate mean ± 1SE. When not shown less than the symbols.

component plant parts. The fraction of nitrogen allocated to roots decreased from 0.20 to 0.15. Within shoots, the fraction allocated to leaves increased from 0.70 at 0 DAT to 0.76 at 507 DAT; corresponding numbers for fractions of nitrogen for branches and stem were quantitatively similar and decreased from 0.15 and 0.12 over the period 0 to 507 DAT.

Photosynthetic characteristics - Series 1

Light response curves of leaf photosynthesis (Figs. 4a and b; Table 1) showed little and inconsistent effects on $A_{\max}$ of the radiation environment the plants were growing in (R50 versus R100). Fertilizer N application enhanced $A_{\max}$ from 4.48 to 6.11 µmol $m^{-2}s^{-1}$ (table 1, variety 74-40) or from 4.01 to 4.75 µmol $m^{-2}s^{-1}$ (table 1, variety 74-110). Overall, in this dataset, $A_{\max}$ of variety 74-40 was 5.3 µmol $m^{-2}s^{-1}$, i.e. about 1 µmol $m^{-2}s^{-1}$ higher than for variety 74-110 (average of 4.4 µmol $m^{-2}s^{-1}$). Initial slopes of radiation response curve, Ø (Figs. 4a and b; Table 1), were statistically similar regardless of treatments. Theta was higher in variety 74-40 than in variety 74-110 (0.59 versus 0.13 mol mol^{-1}). There were three way interactions between radiation levels; varieties and N rate (Table 1). With increasing growth radiation level, R_d increased from -0.46 to -0.88 µmol $m^{-2}s^{-1}$ in variety 74-40 and from -0.48 to -0.69 µmol $m^{-2}s^{-1}$ in variety 74-110. However, Rd was only marginally affected by nitrogen nutrition.

Figs 5a-c explore associations between leaf properties across treatments, in particular SLA, and N_a and their associations with $A_{\max}$. Both N_a (Fig. 5a) and $A_{\max}$ (Fig. 5b) were linearly but inversely associated with SLA. Conversely, N_a and $A_{\max}$ were linearly and positively associated (Fig. 5c). Pooling data across all treatments, $A_{\max}$, was linearly regressed as a function leaf nitrogen content per unit leaf area (N_a). The regression was significant ($p < 0.001$) and $A_{\max}$ could be described adequately by a simple linear regression ($n = 16$, $R^2 = 0.68$, $p < 0.001$; numbers in the bracket represent SE of the coefficients)

$$A_{max} = 1.38\ (0.47) + \ 2.33(0.25)\mathrm{N}_a \qquad \text{(Eqn 5)}$$

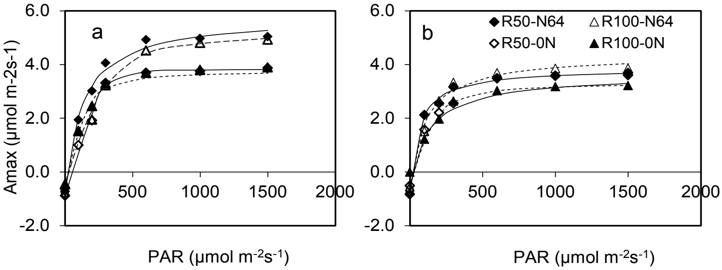

Fig. 4. Photosynthetic light response curve of coffee leaves of two different varieties (a) 74-40 and (b) 74-110 grown in open sun or 50% shade with no or a supply of 64 g tree^{-1} nitrogen fertilizer . Each data point was the average of a minimum of sixteen values taken from 23 September to 17 October 2013 (433 to 447 DAT). Curves were fitted using a non-rectangular hyperbola model (Eqn 3).

Table 1. Radiation and nitrogen effects on $A_{\max}$, θ and ∅ of light response curves fitted to a non-rectangular hyperbola model of photosynthesis (Eqn. 3) for coffee varieties 74-40 and 74-110 grown under different radiation levels and nitrogen nutrition

Treatment	Amax (µmol m^{-2}s^{-1})	θ (mol mol^{-1})	∅	Rd (µmol m^{-2}s^{-1})
R100-N64-74-40 [1]	5.91^{b}	0.49^{c}	0.02^{a}	-0.46^{d}
R100- N0 -74-40	4.26^{de}	0.80^{b}	0.03^{a}	-0.47^{d}
R50-N64-74-40	6.31^{a}	0.11^{d}	0.05^{a}	-0.73^{b}
R50 -N0 -74-40	4.70^{cd}	0.97^{a}	0.02^{a}	-0.88^{a}
R100-N64-74-110	4.94^{c}	0.01^{d}	0.05^{a}	-0.55^{c}
R100-N0-74-110	3.86^{ef}	0.48^{c}	0.03^{a}	-0.48^{d}
R50-N64-74-110	4.56^{d}	0.02^{d}	0.07^{a}	-0.69^{b}
R50-N0-74-110	4.15^{e}	0.02^{d}	0.04^{a}	-0.51^{cd}

[1] see Materials and Methods for explanation of treatment codes. Numbers followed by the same letter in the same column are not statistically different from one another according to LSD (5%).

Photosynthetic characteristics - Series 2

Net rate of carbon assimilation, A_{600}, measured at saturated light intensity (600 µmol m^{-2} s^{-1}) increased with increasing radiation and nitrogen supply, albeit with no statistical differences between R70 and R100 and between N64 and N88 (Fig. 6). The interaction between radiation level and N rate was statistically significant ($p < 0.001$); the positive effect of radiation on A_{600} being somewhat stronger at high than at low N supply. There were some differences between varieties with varieties 74-4 and 74-40 exhibiting significantly higher $A_{\max}$ than variety 74-110. Varieties 74-110 and 74-40 had greater C_i value than variety 74-4. Stomatal conductance and transpiration rates were also different among varieties (Table 2).

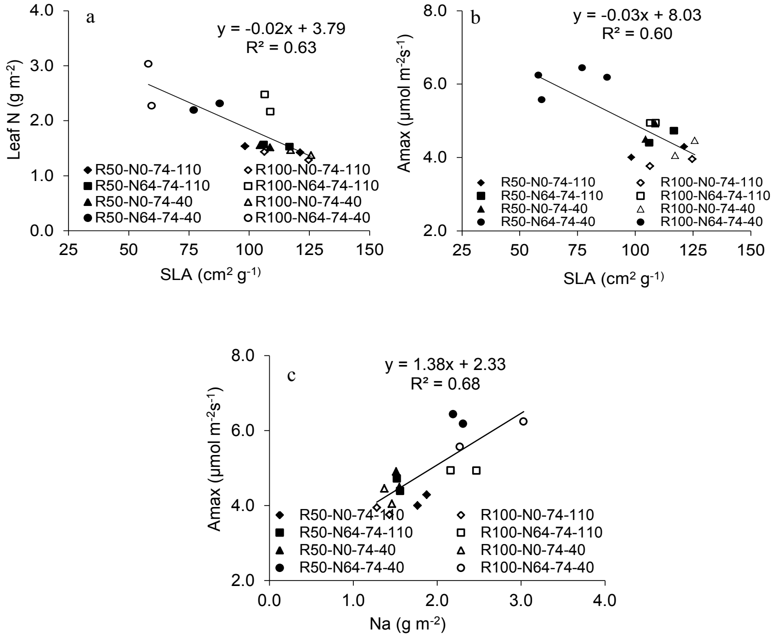

Fig. 5. (a) Nitrogen concentration per unit leaf area, N_a, and (b) light-saturated rate of leaf photosynthesis, $A_{\max}$ (Eqn 3) versus specific leaf area, SLA; (c) A_{max} versus leaf nitrogen concentration per unit leaf area of coffee plants gown in full sun (R100) or 50% shade (R50) in combination with no (N0) or supply of nitrogen of 64 g $tree^{-1}$ $year^{-1}$ (N64). Each data point was mean of three data points averaged across replications taken on October 10-2014 (440 DAT) in three replicates.

Table 2. Varietal effects on A_{600}, g_s (stomatal conductance), and C_i (internal CO_2 concentration) of coffee trees grown under different levels of radiation and rates of nitrogen supply.

Variety	A_{600} ($\mu mol\ m^{-2}s^{-1}$)	g_s ($\mu mol\ mol^{-1}$)	C_i ($mol\ mol^{-1}$)
7-4	4.63^a	0.045^a	221.3^b
74-40	4.60^a	0.041^b	232.9^a
74-110	4.30^b	0.045^a	235.6^a

Different letters in a column indicate statistically significant differences at 5% LSD.

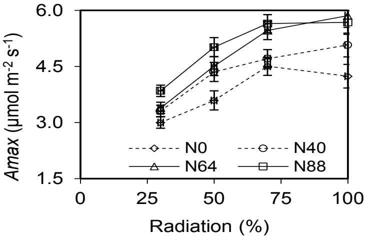

Fig. 6. Effects of different radiation levels and rate of nitrogen supply on light saturated rate of leaf photosynthesis, A_{600}. Each data point is the average of nine values, average across three varieties and three replicates taken on May 16 - 20, 2014 (393 - 397 DAT). Vertical bars indicate ± 1 SE and when not shown, SE was less than the symbol.

Bean yield

Green dry coffee bean yields were positively related to both radiation and N supply. In both cases, the responses were curvilinear; with differences between the highest (N88 or R100) and second highest (N64 or R70) nitrogen and radiation levels, respectively, being non-significant ($p > 0.05$; Figs. 7a and b). The effect of nitrogen supply was stronger at high radiation than at low radiation (i.e. positive interaction between radiation and N supply ($p < 0.05$)). In R30 and R50, yield declined for N supply beyond 64 g tree^{-1}. In R70 and R100, however, yield appeared to monotonously increase at declining marginal efficiency.

The varietal effect on bean yield was significant ($p = 0.02$): across radiation and nitrogen treatments variety 74-40 had a significantly higher bean yield (286.3 g tree^{-1} = 1118 kg ha^{-1}) than variety 74-4 (272.4 g tree^{-1} = 1063 kg ha^{-1}) and variety 74-110 (265.9 g tree^{-1} = 1039 kg ha^{-1}), which latter two did not differ from each other.

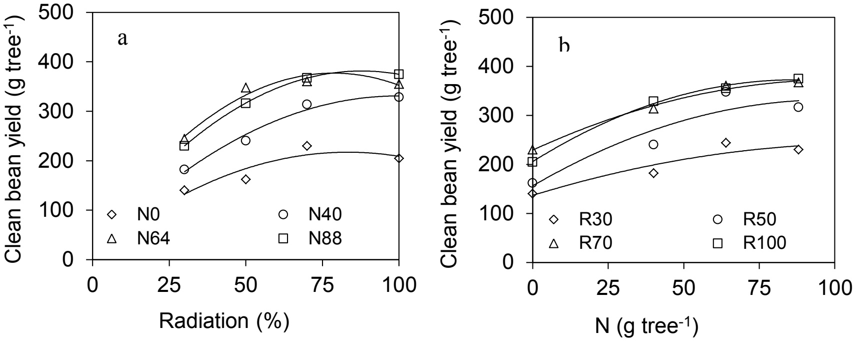

Fig. 7. Average bean yield (ca 11% moisture) of coffee trees plotted (a) against different radiation levels and (b) the annual rate of N supply. Data pertain to the first yield of berries, harvested 10 to 20 December 2014 (872 – 882 DAT;). Data points are means of nine values averaged across varieties. Lines were fitted using quadratic equation: $y = 10.3 + 4.95x - 0.03x^2$, $R^2 = 0.82$; $y = 20.4 + 6.15x - 0.03x^2$, $R^2 = 0.98$; $y = 34.8 + 8.81x - 0.056x^2$, $R^2 = 0.97$; $y = 28 + 8.1x - 0.047x^2$, $R^2 = 0.99$, respectively for 0 to 88 g tree^{-1} N. $y = 136.8 - 0.008x^2 + 1.88x$, $R^2 = 0.87$; $y = 155.4 - 0.02x^2 + 3.60x$, $R^2 = 0.88$; $y = 206.8 - 0.02x^2 + 3.74x$, $R^2 = 0.98$; $y = 206.8 - 0.021x^2 + 4.08x$, $R^2 = 0.98$, respectively, for 30 to 100% radiation levels.

Discussion

The experiment included rates of N supply covering the range from severe limitation to saturation of the response to N, in combination with a large span of degree of shading, at least covering the degrees of shade that are commonly manifest in managed coffee systems. Figs. 1a-d represents component panels of the ‘three quadrant diagram for nitrogen response’ (de Wit 1953; Hartemink et al. 2000; van Keulen 1982). Fig. 1a answers the question ‘how much plant biomass is returned per unit of N input’. Such relations are of agronomic importance as is Fig 7 showing the response of yield to N supply. Slopes and intercepts of relations in Fig 1a and Fig. 7 differ depending on environmental and physiological factors. One of these determinants is the ’natural’ productivity, i.e. the production at zero N input. Fig. 1b underpins how treatments affect the relation between supply and uptake of N, i.e. the effect of factors and processes in the domain of the soil (including mineralization, immobilization, leaching, denitrification and volatilization) and distribution and uptake activity of roots. Figs 1c and 1d show the ‘conversion’ from N taken up into plant dry matter; differences in this ‘conversion’ are related to differences in physiology of the plant. For a given N application, radiation improved biomass production, enhanced the agronomic response to N (Fig. 1a). Fig 1b underlines that treatment effects on the ’physiology’, that is the ‘conversion’ of a unit of N taken up into plant dry matter, is conservative. Figs 1c and 1d indicate that most of the variation across treatments in the agronomic response is due to variation in the relation between supply and uptake. Larger radiation interception per plant enlarges the carbon resources for growth and enlarges the requirement for N. When sufficient N is available, the growth potential, set by the radiation level, can be realized.

Coming back to Figs 1c and 1d: there was some modulation of the association between biomass and N uptake by radiation (Fig. 1c) and nitrogen (Fig. 1d). Such modulations are explained from changes in distribution of dry matter and nitrogen across treatments and by luxury N uptake at high N supply. The regression equation between dry matter and N uptake can be applied to estimate N content from biomass or calculate required uptake to produce a given amount of biomass.

Apparent nitrogen recovery, ANR is the slope of the response in Fig. 1b and ranged from 7 to 17 % across treatments. These values are very low compared to those generally reported in crops, e.g. potato (Vos and Van der Putten 2001), which are typically ranging from 34 to 65 %. Notably such high values have also been reported for coffee. For instance, Bruno et al. (2014) found 60 % of the applied N in standing plant material plus another 21% in litter underneath the tree, most of which was probably derived from plant material. However, other studies on coffee found low ANR values that were comparable to ours: e.g. Fenilli et al. (2007); (2008) reported 19 % and Cannavo et al. (2013) 13.5 %. Also in other tree species low ANRs were reported, e.g. 6.3 % for young pear (Neto et al. 2006) and 8.3 - 12.7 % for peach (Nario et al. 2003). This raises the question as to why such low values may occur. One aspect is perhaps that N immobilization is rapid in tropical environments (Dourado-Neto et al. 2010), probably resulting in more N to be preserved in soil colloidal material (Bruno et al. 2014; Moreira et al. 2006), or perhaps due to slow process of root growth that are observed in most young plants (Neto et al. 2006; Neto and Matsumoto 2010).

The physiological efficiency, PE declined with increasing N supply due to increase in N concentration in the whole plant or plant organ (e.g. leaves). Such decrease in PE with increasing nitrogen supply was also reported for different crop species e.g. rice (Buresh et al. 1988), oats (Isfan 1993) and other cereal crops (Tilman et al. 2002), and probably reflects the fact that as nitrogen uptake is increased it tends to become less limiting as growth increasingly becomes constrained by other factors.

The current study shows light saturation of leaf photosynthesis rate at 300 - 600 µmol m^{-2} s^{-1}, corroborating observations by Fahl et al. (1994), justifying measurement of A_{max} at

600 μmol m^{-2} s^{-1}. The (larger) dataset of 'series 2' showed higher A_{max} with more radiation (Fig. 6). Most of these differences in A_{max} are associated with an effect of radiation on N_a (Figs. 2b and 5c; Chapter 2). High radiation plants exhibit high N_a and thus A_{max} (e.g. Anten et al. 1996). Positive effects of nitrogen on photosynthetic capacity are also primarily explained from higher N_a (Anten et al. 1996; Evans 1983; Seemann et al. 1987). Though nitrogen did not affect SLA (Chapter 2), part of the lower A_{max} in low-radiation environments are due to an additional reducing effect of higher SLA. Ample N improves RUE (Chapter 2) with larger positive nitrogen effects the higher the radiation levels. N-limited coffee exposed to full sun is prone to photo damage, but ample availability of N triggers photo protective mechanisms (Fahl et al. 1994; Ramalho et al. 1999; Ramalho et al. 2000).

The yields obtained in this study were in the range of yields green beans of 90 to 480 g $tree^{-1}$ that Arabica coffee can potentially produce (Bruno et al. 2011; Campanha et al. 2004; Haggar et al. 2011; Lin 2009; Obso 2006; Peeters et al. 2003). Both shading and nitrogen limitation significantly reduced coffee bean yield (Fig.7). The results are in agreement with the common observation that coffee bean yield increases with radiation (Haggar et al. 2011; Jaramillo-Botero et al. 2010) and with nitrogen fertilizer application (Endris et al. 2008; Pompelli et al. 2010). We presented only the first year of bean production. Bean harvesting entails extraction of resources from the system; higher yields at high radiation thus entail a greater loss of N. It is therefore possible that the positive interaction between N and radiation availability would be stronger when long-term yields are considered. Yields per tree, y (g), were associated with biomass at 507 DAT, x (g), as given by the regression equation $y = 11.22 (25.23) + 0.29 (0.03) x$, ($n = 16$, $R^2 = 0.89$, $p < 0.001$). This association underlines the importance of unrestricted early vegetative growth for bean yield.

In conclusion, biomass production and yield of coffee trees are the results of the interactive response of the trees to both radiation and nitrogen fertilizer rates. The availability of the two factors plays a pivotal role in coffee tree growth and productivity. We demonstrated that more N was utilized and more biomass and yield were produced the more radiation was available. The association between biomass and uptake can be applied to estimate N content from biomass or calculate required uptake to produce a given amount of biomass. Such relationships will also be useful to improve allocation rules in a process-based growth model of coffee trees. Coffee trees showed low apparent nitrogen recovery. Achieving synchronies between N supply and coffee trees demand without excess or deficiency therefore requires further investigation of options to improve the low nitrogen recovery of the trees.

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Chapter 4

Branch growth dynamics, photosynthesis, yield and bean size distribution in response to fruit load manipulation in coffee trees

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Abstract

Imbalance in fruit load and branch growth plays a role in the occurrence of biennial bean production patterns in coffee (*Coffea arabica* L.). Effects of fruit load manipulations were studied in two field experiments in the Jimma region, Ethiopia, over two consecutive years. Treatments consisted of reducing fruit loads in the pinhead stage to 25, 50, 75 % and controls keeping 100 % of the fruits per tree (treatments coded T25 through T100). Treatments were applied in the first year only. Branch growth and the formation of new leaves, drop of old leaves, light saturated rate of leaf photosynthesis ($A_{\max}$), nitrogen content of leaves on selected branches, as well yield and bean characteristics were evaluated throughout the experimental period. The study revealed that branch growth, and leaf N content per unit leaf area, N_a, were inversely associated with fruit load, whereas loss of basal leaves on branches increased with fruit load and over time. $A_{\max}$ was strongly and linearly associated with N_a and declined with increase in fruit load. In the year of treatment green bean yield increased with fruit load, but in the second year the reverse was true. On aggregate over two years, treatment T25 and T50 out yielded treatments T75 and T100. Fruit thinning shifted the bean size distribution to larger sizes. In conclusion, fruit thinning modulated the balance between branch growth and fruit development. Thus enhanced branch growth, improved bean size and stabilized yield over years.

Key words: Fruit thinning, $A_{\max}$, bienniality, assimilate, berry, pinhead

Introduction

Like other perennial fruit trees, e.g. olive (*Olea europaea* L.) (Dag et al. 2010; Lavee 2007), apple (*Malus domestica* Borkh.) and a host of other species (Guitton et al. 2011) coffee is prone to biennial bearing. Biennial bearing (alternate bearing) means that years with high fruit yields alternate with years with low fruit yield. Both commercially dominant coffee species *Coffea arabica* L. (Cannell 1985) and *Coffea canephora* Pierre ex Froehner, syn. (Rodrigues et al. 2013) are prone to biennial bearing. However, the phenomenon is more pronounced in *C. arabica* than in *C. canephora*. In *C. arabica*, bienniality occurs in all coffee cultivation systems, but its manifestation is more severe in open sun than in shaded systems (DaMatta 2004).

Coffee plants tend to flower heavily (DaMatta et al. 2008), resulting in the production of high fruit loads. A high fruit load demands proportionally more resources (assimilates and nutrients) (Vaast et al. 2001), which could otherwise be used for new vegetative growth. In coffee appearance of new branches and extension of the existing ones are essential as new growth provides the nodes for new flower bud positions in the next season. In C. *arabica,* active growth of the branches proceeds with the onset of rain (DaMatta et al. 1999; Partelli et al. 2013; Rena et al. 1994). The branch apex initiates additional phytomers (units consisting of leaves, node, internode and lateral bud). On plagiotropic branches (Cannell 1985) buds develop into flower clusters or grow out into lateral branches. Flowering occurs at some distance, i.e. number of phytomers, from the apex. On a branch the zone with flowers and fruits moves in apical direction; at tree level, flowering and fruiting move from lower to higher branches. Flowering of sufficiently developed flower buds is triggered by rain or temperature drop (Rena et al. 1994). In a zone with buds 'ready to flower' fractions of flower buds may open sequentially upon several inductive events (DaMatta et al. 2007) giving rise to an extended period of harvest. Without new growth, coffee trees do not produce fruit since flower buds are only produced once on a specific segments of plagiotropic branches (Arcila-Pulgarín et al. 2002; Rena et al. 1994). In short, reduced vegetative growth reduces potential bean production in the next year. Imbalance in fruit load and vegetative growth is seen as the basis of biennial bearing rhythms (Cannell 1985).

Severe imbalance between sink demand by growing fruits and source supply may even result in 'dieback', i.e. the progressive death of branches from their apex in a basipetal direction (DaMatta et al. 2007). Dieback reinforces bienniality. Bienniality is undesirable from the viewpoint of coffee growers, because it causes not only highly fluctuating yields but also fluctuating bean sizes. Causes and consequences of bienniality may differ among fruit tree species. In apple, biennial bearing is attributed to competition between initiation of flowers for the next season and current fruit formation (Guitton et al. 2011). In olive, it is controlled by the interaction between vegetative growth and fruit load. During high fruit production vegetative terminal buds are even inhibited (Lavee 2007). In coffee, however, the dynamics of mechanisms causing bienniality are still poorly understood and the means of adequately managing it are currently lacking.

Fruits act as priority sinks and dry matter allocation to them during the bean-filling stage may be more than four times that allocated to branch growth (Vaast et al. 2006). At this stage, more than 95% of N, P and K absorbed by the plant is accumulated in fruits and beans (Cannell 1985). Feedback was also shown between the degree of carbohydrate accumulation and leaf photosynthesis (Franck et al. 2006; Vaast et al. 2005). Franck et al. (2006), stated that the maximum rate of leaf photosynthesis, $A_{\max}$ was negatively correlated with the total soluble sugar concentration (SSCm) in leaves. $A_{\max}$ increased with fruit load on a girdled branch and decreased during the day, indicating that limiting sink demand for carbohydrates caused SSCm to accumulate in the leaf tissue. This resulted in down-regulation of $A_{\max}$. Other studies also observed regulation of photosynthesis by the level of photoassimilates and so by fruit load in coffee (DaMatta et al. 2008; Vaast et al. 2005).

Manipulation of the fruit load has been applied as a measure to improve fruit quality and as a research tool to gain insight in the competition between shoot growth versus flowering and fruit growth. In Ethiopia, fruit load management is not practiced at all in coffee. The current study explores effects of fruit load manipulation as a first step towards proposing practical management options. Some previous studies manipulated fruit load by thinning fruits on selected branches, assuming that the conclusions are valid for a whole tree (Franck et al. 2006; Vaast et al. 2005). However, lack of branch autonomy and movement of assimilates from branches with lower fruit load to those with higher fruit load has been reported (Chaves et al. 2012). Thus, it may not be appropriate to scale up fruit thinning effects from individual branches to the whole plant level. In the current study, fruit thinning was applied to all branches of a tree to test the following hypotheses: (1) within a given year, thinning of young fruits modulates resource allocation between fruit production and branch growth. Fruit thinning decreases fruit production freeing up resources to enhance branch growth, and hence, sites for future flower production. This reduces biennial bearing and stabilizes yields over years. (2) In coffee trees with high fruit load, the assimilate demand by fruits and vegetative organs enhances light saturated leaf photosynthesis so that more carbohydrates are produced when needed. (3) Different degrees of fruit thinning will reveal the degree of coffee bean plasticity (in size and weight); that is, different degrees of fruit thinning will reveal how plastic the beans are in size and weight.

To test these hypotheses, two experiments were conducted at two sites with shade-grown coffee trees. Fruit thinning (trees with 0, 25, 50 and 75 % of the fruit removed) was applied and data on branch growth, leaf photosynthesis, yield, bean size and bean dry weight were collected for two consecutive years (no fruit load manipulation in the second year).

Materials and Methods

Description of experimental sites and treatments

The study was carried out in two consecutive years in two experiments on Arabica coffee at two different sites. The first experiment (Expt I) was conducted from 7 June 2012 to November 2013 at 'Gomma-I' coffee plantation site, located at 1570 m elevation, 7^o 57' N, 36^o 42' E, with annual mean temperature and rainfall of 20.5 °C and 1600 mm, respectively. The soil of the area was characterized as a Eutric Nitosol type, with pH ranging from 4.5 – 6.0 (Mekonnen and Belehu 2007). The second experiment (Expt II) was conducted from 14 May 2013 to November 2014 on a farmer's field located in Mana district of Jimma zone, Ethiopia. The site is located at an elevation of 1750 m, 8^o 67' N, 37^o 07' E, with an annual average temperature of 20.5 ^oC and rainfall of 1525 mm. The soil of the area was characterized as a Nitosol type, with pH ranging from 4.5 to 5.5.

In both cases, eight-year old Arabica coffee trees of cultivar 74-40 (resistant to coffee berry disease), planted under *Albizea spp*. shade trees (35 – 45% shade), at a spacing of 2.0 x 2.0 m between coffee plants and rows, were selected and used for the experiments. Sixteen coffee trees with comparable size and morphology were selected for the study. In Expt I plants had 10-12 pairs of fruit bearing primary plagiotropic branches per plant, 20 - 22 fully expanded individual leaves per primary branch, 130 - 140 fruits per branch; in Expt II there were 10 – 12 pairs of fruit bearing primary branches per plant, 10 – 15 individual leaves per branch and 90 – 130 fruits per branch. Plants were selected from four adjacent plant rows and randomly assigned to the different treatments in a completely randomized block design with four replications, while taking care that the starting size of trees did not differ significantly among treatments. Following this selection, four fruit load treatments were manually imposed on all fruit bearing branches of the plants on 7 June 2012 (Expt I) and 14 May 2013 (Expt II). No fruits were removed in the control treatment while 75, 50 or 25% of the fruits were removed from each node of the branches in the other three treatments resulting in four treatments namely T100, T75, T50 and T25. When treatments are expressed as leaf area (measured as described below) at the time of thinning to fruit ratio counted upon thinning, the values were 8.9, 12.6, 21.2 and 38.8 in Expt I; 6.0, 7.6, 11.5 and 22.0 cm^2 per fruit in Expt II, respectively for T100, T75, T50 and T25. For adequate resource supply to fruits a minimum ratio of 20 cm^2 per fruit has been proposed by Cannell (1985). In both experimental sites, there were no disease and pest problems. In Gomma coffee plants received 150 kg N $ha^{-1}yr^{-1}$ whereas coffee grown in the farmer's site was organic, receiving compost and manure alone.

Measurements

Growth measurements

For evaluation of vegetative development of the trees over time, expressed in days after fruit thinning, DAFT, four plagiotropic branches per tree positioned in the main producing zone of the canopy were selected and tagged. Starting at the time of imposing the treatment, non-destructive measurements of branch length (cm), branch diameter at its base (cm) and leaf number per branch were made three times (for Expt I) from the pinhead stage (0 DAFT) to fruit harvesting (225 DAFT) and five times (for Expt II) from pinhead (0 DAFT) to fruit ripening (169 DAFT).

For destructive leaf area and dry weight evaluations, in Expt I fully developed third and fourth pairs of leaves, counted from the apex, were tagged at the time of thinning on the branches immediately opposite to the branches selected for non-destructive monitoring of leaf dynamics. Out of these leaves, and leaves in similar position from adjacent branches, three to five leaves were randomly detached at every sampling date to determine leaf area and dry weight. In Expt II, branches opposite to the selected branches were also tagged at their apex (tags indicated the most recently formed leaves) to monitor the effect of fruit thinning on leaf area and dry weight of subsequently developing new leaves. For the first sampling, the first and second leaves, counted from the apex and distal to the tagged position, were taken to the laboratory. After fruit thinning, at every sampling date, leaves from leaf insertion number one, counted from the tagged position to the apex from each branch and each treatment, were sampled. After each sampling date, the tag position was moved to the tip of the same branch so as to separate newly emergent leaves from already appeared ones. In the laboratory, leaf area of sampled leaves was measured, using a leaf area meter (AM- 200, Delta T devices Ltd., Cambridge, UK); leaves were dried in an oven at 70 ^{o}C for 72 h to a constant weight and their dry weights were determined using precision balance (model CTG-6H, Citizen scale plc., Parwanoo, India) with precision 0.01 g.

Leaf gas exchange variables

In Expt II the net rate of carbon assimilation ($A_{\max}$, in µ mol CO_2 m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i, in mmol mol^{-1}) were measured in leaves from all treatments at 43 DAFT (bean filling) and 153 DAFT (fruit ripening) (Arcila-Pulgarín et al. 2002). These measurements were made on fully developed leaves that were part of the third pair counted from the apex of the fourth pair of plagiotropic branches, counted from apex of the selected orthotropic trunk, using a differential CO_2/H_2O infrared gas analyser LCpro+ (ADC BioScientific, Hoddesdon, U.K.). Series of measurements were taken from 09 – 13 h. Recordings were made after 15 to 20 min acclimation time at a saturating incident radiation of 600 µmol m^{-2} s^{-1}, chamber temperature of 27± 3°C and ambient CO_2 (370 - 380 µ mol mol^{-1}) and relative humidity (70 – 80%). After completion of the gas exchange measurements, leaves were cut off and taken to the laboratory for determination of their leaf area, dry weight and nitrogen concentration in the dry matter. Total nitrogen concentration in the dry matter was determined by Kjeldahl method after submitting the plant material to oxidation by sulphuric digestion (Horneck and Miller 1998; Jones 1991).

Fruit attributes

Only fully ripe, red coloured coffee berries were harvested from each individual tree. Three harvests were conducted at one-week intervals to completely harvest all fruits ripening at different times. Fresh weight of berries at each harvest was recorded before processing. Coffee fruit samples were subjected to wet processing and dried according to standard agronomic practices to a moisture content of ca. 11%. Bean weights (g) of the dried beans were determined in three replicates and the result was indicated on individual bean weight base. Bean diameters (mm) were measured on 80 randomly taken beans per treatment, using a digital calliper (Mitutoyo, IP 67, CD-20-PPX, Kawasaki, Japan).

Statistical analysis

Data on each sampling date were statistically examined using a fixed-model one-way ANOVA following a completely randomized design. Significant differences between treatments were analysed using LSD test at P ≤ 0.05. All of the statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). To test the significance of differences in A_{max} among the different fruit load treatments, we performed analysis of covariance (ANCOVA) model including leaf nitrogen content as a covariate, A_{max} as dependent variable and fruit load as categorical factor.

Results

Branch growth

Over the experimental periods, increments in length and diameters of branches were inversely associated with fruit load (Figs. 1a – d). In Expt I differences in branch length increased between 50 – 150 DAFT, while the differences in length did not increase further beyond 150 DAFT (Figs. 1a and b). Branch length increment over the experimental period was 34 cm in T25 and 19.7 cm in T100, i.e. a difference of almost 50 %. Similarly for Expt II increases in branch length were 15.0 cm in T25 and 4.0 cm increase in T100, i.e. a difference of 73%. In Expt II differences among treatments in branch length increased over the whole experimental period (Figs. 1c and d).

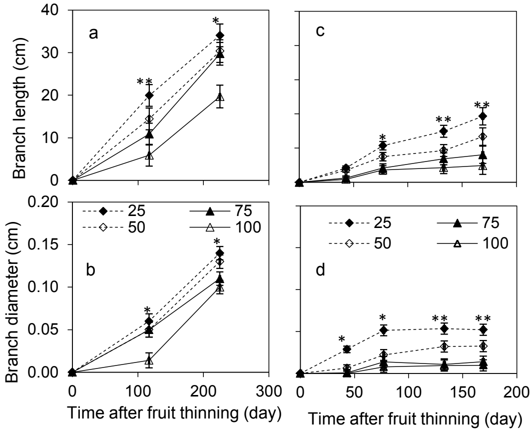

Fig. 1. Effect of fruit load on branch length (a, c) and branch diameter increments (b, d) of field grown coffee trees. Panels a and b are data from Expt I and c and d are from Expt II. In the legend the numbers indicate the fruit thinning treatments. Vertical bars indicate mean ± 1 SE. When not shown, SE was smaller than the symbol. *, ** indicate significance differences between treatments, respectively at $P < 0.05$ and $P < 0.01$

In both experiments loss of leaves from basal positions of the branches generally increased with fruit load and over time. In T100 (10.66 fruits cm^{-2}) of Expt I, on average 5.35 leaves, constituting about 25 % of basal leaves of branch under consideration, were lost over the experimental period. Conversely, in T25 dropping of leaves started after 117 DAFT and amounted to 0.75 leaves on average, i.e. only 3.4 % of the basal leaves, at the end of the experimental period (Fig. 2a). In Expt II the pattern of loss of basal leaves was similar to the one observed in Expt I with 3.51 leaves lost in T100 and none in T25 (Fig. 2b).

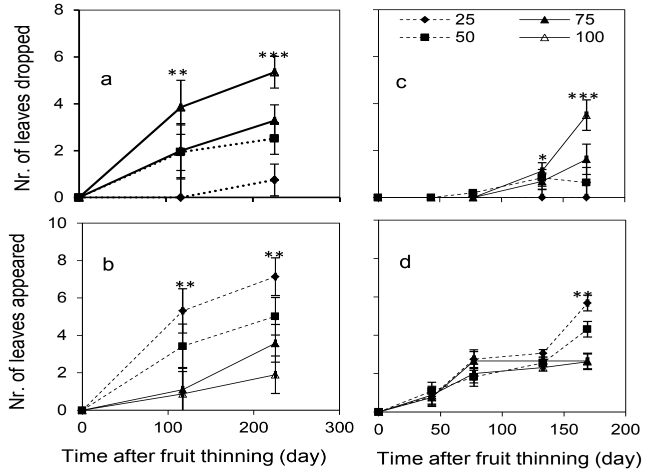

Fig. 2. Cumulative number of basal leaves dropped (a and c) and apical new leaves appeared (b and d) during the experimental periods for coffee trees with different fruit load. All data points are averages of four values and were taken from Expt I (a, b) and Expt II (c, d). Vertical bars indicate mean ± 1SE. *, ** and *** indicate significance differences between treatments, respectively at $P < 0.05$, $P < 0.01$ and $P < 001$

In both experiments leaf production at the apex increased with the level of fruit thinning (Figs 2c and d). At the end of the experimental periods the total number of leaves were between two (Expt II) and almost four (Expt I) times larger in T25 than in T100. These observed dynamics of loss and gain of leaves resulted in a large difference in the total actual leaf number per branch at the end of the experimental period: 29.1 and 17.7 for T25 and T100, respectively in Expt I; and 19.3 and 11.8 for T25 and T100 in Expt II (Table 1).

Leaf growth dynamics

Across treatments and sampling dates mean area per leaf ranged between 53 and 70 cm^2 (Table 1). In Expt I at 225 DAFT area per leaf declined with increasing fruit load, and ranged from 70.2 cm^2 in T25 to 52.6 cm^2 in T100. Similar effects of fruit load on final leaf area were observed in Expt II. In both experiments differences between treatments in leaf area increased with time, though this pattern was more clearly discernible in Expt II than in Expt I as there were more measuring points in the former experiment (Table 1).

Table 1. Effect of fruit load treatments (Expt I, 2012/13; Expt II, 2013/14) on net leaf number per branch, area per leaf, leaf dry weight per leaf and specific leaf area of field grown coffee trees at zero, 117 and 225 days after fruit thinning (DAFT) for Expt I and at zero, 43, 77, 133 and 169 DAFT for Expt II.

	Time (DAFT)		Treatments			
Expt		Parameters	T25	T50	T75	T100
I	0	net leaf number	22.6^{a*}	24.0^{a}	20.8^{a}	22. 0^{a}
		leaf area (cm^2)	61.2^{a}	60.1^{a}	58.4^{a}	60.8^{a}
		leaf dry weight (g)	0.41^{a}	0.40^{a}	0.39^{a}	0.41^{a}
		SLA (cm^{2}g^{-1})	148^{a}	150^{a}	149.7^{a}	148.3^{a}
	117	net leaf number	27.3^{a}	23.8^{ab}	21.9^{bc}	18.8^{c}
		leaf area (cm^2)	65.3^{a}	64.8^{a}	62.4^{ab}	59.5^{b}
		leaf dry weight (g)	0.44^{a}	0.43^{a}	0.41^{ab}	0.38^{b}
		SLA (cm^{2}g^{-1})	149.7^{b}	149.0^{b}	151.6^{ab}	156.1^{a}
	225	net leaf number	29.1^{a}	26.2^{ab}	23.1^{b}	17.7^{c}
		leaf area (cm^2)	70.2^{a}	61.2^{a}	57.9^{ab}	52.6^{b}
		leaf dry weight (g)	0.55^{a}	0.48^{ab}	0.45^{b}	0.36^{c}
		SLA (cm^{2}g^{-1})	127.9^{b}	127.8^{b}	123.8^{b}	143.9^{a}
II	0	net leaf number	13.6^{a}	13.7^{a}	13.4^{a}	13.3^{a}
		leaf area (cm^2)	46.0^{a}	46.0^{a}	46.8^{a}	46.0^{a}
		leaf dry weight (g)	0.33^{a}	0.33^{a}	0.34^{a}	0.33^{a}
		SLA (cm^{2}g^{-1})	138.3^{a}	139.4^{a}	137.8^{a}	139.3^{a}
	43	net leaf number	14.4^{a}	14.6^{a}	14.5^{a}	14.1^{a}
		leaf area (cm^2)	34.2^{a}	34.2^{a}	31.9^{b}	30.8^{b}
		leaf dry weight (g)	0.28^{a}	0.28^{a}	0.26^{b}	0.25b
		SLA (cm^{2}g^{-1})	121.3^{a}	123.6^{a}	124.6^{a}	126.4^{a}
	77	net leaf number	16.4^{a}	15.5^{a}	15.7^{a}	15.3^{a}
		leaf area (cm^2)	29.3^{a}	27.1^{ab}	25.7^{b}	25.8^{b}
		leaf dry weight (g)	0.40^{a}	0.37^{a}	0.26^{b}	0.25^{b}
		SLA (cm^{2}g^{-1})	72.8^{b}	75.2^{b}	99.3^{a}	103.2^{a}
	133	net leaf number	16.7^{a}	16.1^{ab}	15.0^{ab}	14.1^{b}
		leaf area (cm^2)	30.5^{a}	30.1^{a}	29.7^{a}	29.0^{a}
		leaf dry weight (g)	0.34^{a}	0.32^{ab}	0.30^{bc}	0.28^{c}
		SLA (cm^{2}g^{-1})	92.5^{b}	100.4^{a}	103.2^{a}	103.5^{a}
	169	net leaf number	19.3^{a}	16.6^{ab}	14.4^{bc}	11.8^{c}
		leaf area (cm^2)	30.3^{a}	25.6^{b}	23.3^{b}	23.16^{b}
		leaf dry weight (g)	0.28^{a}	0.24^{b}	0.22^{b}	0.22^{b}
		SLA (cm^{2}g^{-1})	108.4^{a}	105.8^{a}	105^{a}	106.4^{a}

*Different letters in the same row indicate significant differences among means for each parameter using LSD at $p < 0.05$ level of significance. Analysis of variance was done separately for each sampling date

In Expt I dry weight per leaf ranged from 0.36 to 0.55 g across treatments and samplings. Compared to the initial leaf dry weight at zero DAFT (0.40 g) dry weight increased over time in all treatments except for T100 where it declined to 0.36 g. At the end of the experimental period, differences among all treatment means were statistically different except for the difference between T50 and T75. In Expt II dry weight per leaf ranged between 0.22 and 0.34 g across treatments and samplings. At each sampling date, dry weights per leaf showed the ranking: T25 > T50 > T75 > T100, with increase in the number of statistically significant differences among treatments the later in time the sampling took place. In the last sampling at 169 DAFT all treatment means were statistically different from each other except for T75 and T100 which were not different from one another.

In Expt I, specific leaf area, SLA, ranged between 128 and 155 cm^2 g^{-1} across treatments and samplings. Within sampling dates, there was a tendency towards larger SLA the higher the fruit load, but treatment means did not differ significantly up to 117 DAFT (Table 1). At the end of the experimental period (225 DAFT), SLA ranged from 144 for T100 to 128cm^2 g^{-1} for T25, the mean of T100 being significantly different from all other treatment means.

SLA of leaves sampled in Expt II ranged from 73 to 139 cm^2 g^{-1} across treatments and samplings. At 43 DAFT, no substantial differences were observed among treatments. Between day 77 and 169, however, leaves of T100 had significantly greater SLA (103cm^2 g^{-1}) than corresponding leaves in T25 (73 cm^2 g^{-1}). Between 0 and 169 DAFT SLA decreased from 139 to 106 cm^2 g^{-1} in T100 and from 138 to 107 cm^2 g^{-1} in T25 with no statistically significant differences among any of the treatment means.

Gas exchange and leaf N content

There was a large effect of fruit thinning on gas exchange variables, measured in Expt II (Figs. 3a and b). Measurements made at 49 DAFT (bean filling stage) and 153 DAFT (ripening stage) showed the same results. $A_{\max}$, the light saturated rate of leaf photosynthesis, ranged between 4.10 and 4.74 µmol $m^{-2}s^{-1}$ across treatments and sampling dates and tended to decrease rather than increase with percentage fruit load (expressed as crop load value, cm^{-2}). No significant differences were observed in the light saturated rate of leaf photosynthesis neither between T25 and T50 nor between T75 and T100 at both sampling dates, but the differences were statistically significant between the two treatment groups (Fig. 3a). Internal carbon dioxide concentration, C_i, on the other hand, increased with fruit load. Leaves of coffee trees thinned to 25% fruit load had significantly lower internal carbon dioxide concentration (233 μmol mol^{-1}) than leaves of trees with full fruit load (250 μmol mol^{-1}) (Fig. 3b). Leaf N content (amount of N per unit leaf area, Na), measured in leaf samples used for gas exchange variables, decreased with fruit load, ranging from 1.86 to 2.31 g m^{-2} in the T100 treatment and from 2.59 to 2.71 g m^{-2} in the T25 treatment. The association between $A_{\max}$ and leaf nitrogen showed a strong and positive linear association (ANCOVA $P < 0.03$; Fig. 4).

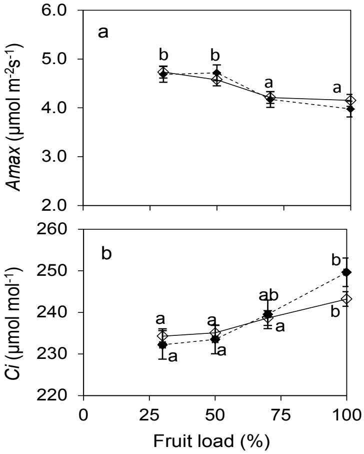

Fig. 3. Effect of fruit load on light saturated rate of leaf photosynthesis, $A_{\max}$ (a) and internal carbon dioxide concentration, C_i (b) of field grown coffee trees. Measurements were made in Expt II at two stages of fruit development: bean filling 43 DAFT (solid lines) and fruit ripening 153 DAFT (dashed lines). Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. Means with the same letter on the same line are not significantly different according to LSD ($P = 0.05$).

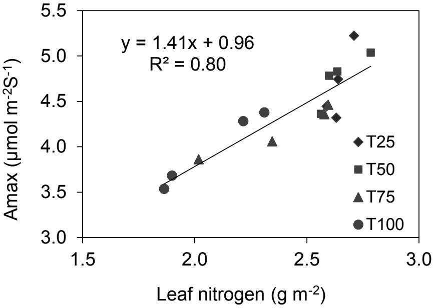

Fig. 4. Relationships between light saturated rate of leaf photosynthesis ($A_{\max}$) and leaf nitrogen content per unit area (N_a). Data points are from all fruit load treatments (means of three replicates) of Expt II collected at fruit ripening stage (153 DAFT)

Yield

The comparison between pinhead stage fruit numbers at the time of thinning with fruit numbers at harvest indicated that the percentage of fruit dropped increased linearly with fruit load, increasing on average from 0% in T25 to about 25 % in the T75 and 28% in T100 treatments (Fig. 5), consistent in both experiments. Green dry bean yield per tree ranged from no yield to 1100 g across experiments, years and treatments (Figs. 6a and b). Yield increased

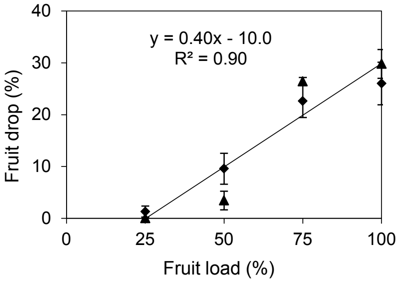

Fig. 5. Percentage of fruit drop, defined as: (the number of harvested fruits per tree divided by the numbers of fruits in pinhead stage left at thinning)*100, as function of initial fruit load. Data points are means of four replicates collected from each coffee tree from both Expt I (triangle) and Expt II (diamonds). Vertical bars indicate SD for trees in each group. When not shown, SD was smaller than the symbol

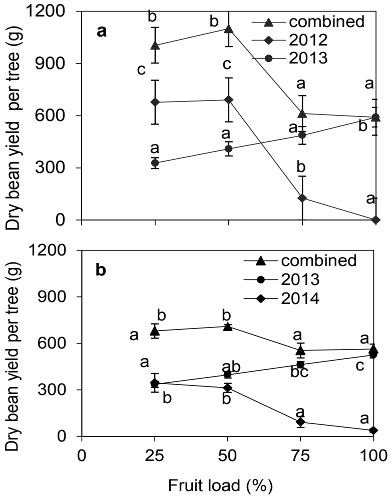

Fig. 6. Mean bean yield per tree as a function of fruit load. Data points are means of four replicates collected from each coffee tree of Expt I (a) and Expt II (b). Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. Means with the same letter on the same line are not significantly different according to LSD ($p = 0.05$).

linearly with fruit load in the first experimental year. In the second year, yield strongly declined with fruit load in the first year; in T100 and T75 there was little or no yield in the second year. Aggregating the yield of the two years showed that T25 and T50 out yielded T75 and T100 by an average of 450 g per tree or 43 % in Expt I. Corresponding numbers for Expt II were 108 g per tree or 15 %.

Fruit thinning, significantly improved individual weight and size of coffee beans (Figs. 7a and b). Compared to beans in T100, mean dry weight of individual beans in T25 increased by 21 and 22% in Expt I and II, respectively. Similarly, bean size (diameter) increased by 14 and 11% when fruits were thinned to 25% of the full load, respectively in both Expts I and II. This increased the percentage of medium and larger bean sizes (bean size > 6.0 mm) in T25 compared to the percentage in T100. The data also showed larger relative variability in bean weight and bean size for beans of coffee trees with higher fruit load than in trees with lower fruit load (Figs. 7c and d).

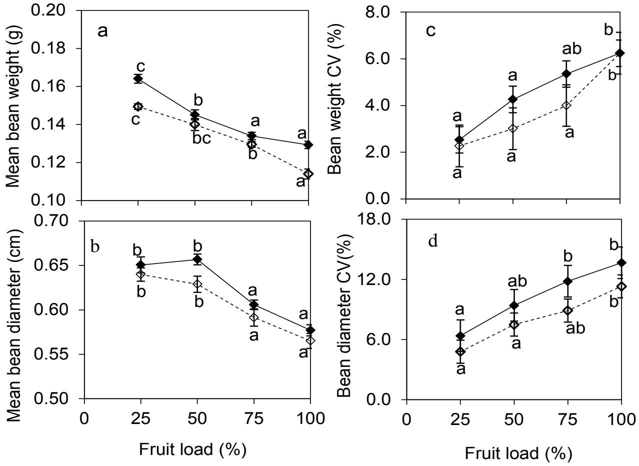

Fig. 7. Mean weight (a) and diameter (b) of dry coffee beans with their corresponding relative variability (c) and (d), respectively versus fruit load. Data points are means of four samples with 1000 seeds each and 80 for diameter collected from coffee tree of each treatment in Expt I (solid lines) and Expt II (dashed lines). Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. Means with the same letter on the same line are not significantly different according to LSD ($p = 0.05$)

Discussion

Fruit production versus vegetative growth and properties of vegetative organs

This study analysed the effect of fruit thinning on vegetative growth, leaf photosynthesis, bean yield and bean size distribution in coffee. Fruit thinning strongly modulated fruit and branch growth. By removing fruits at a very young stage, branch growth and production of additional apical leaves were stimulated while shedding of existing leaves was reduced. Not only numbers of organs were affected (Table 1, Fig. 2), also their properties such as area, weight and specific area of leaves and diameter of branches (Table 1, Fig 1,). All these changes are consistent with, but not conclusive proof of the view that more substrates are available to maintain and expand vegetative organs at low fruit load than at high fruit load. In this context, it is also significant and new information that the degree of leaf shedding increased with fruit load (Fig. 2). Bean yields in the year of thinning were evidently reduced, thinned plants overcompensated for this in the second year, such that yield aggregated over two years yield increased with thinning (Fig. 6). These results were very consistent across the two experiments conducted at two different sites and in two different series of years, indicating that the results were representative for similar coffee production systems with shaded coffee trees, not subjected to pruning.

Some of the previous studies compared intact controls and completely defruited trees and reported a reducing effect of the presence of fruits on vegetative growth (Amaral et al. 2001; Cannell 1971; DaMatta et al. 2008). The study of DaMatta et al. (2008), included two treatments comparable to the ones in the current study, i.e. a control treatment and a treatment with 50 % of fruits removed from the whole tree in the pinhead stage. The current result confirms and expands their findings on the competition between branch growth and fruit growth as well as effects on yield in the first year. No previous study examined the strong effects of thinning on yield in the second year after thinning. The higher and more stable yields over two years in T25 and in T50 than in T75 and T100 indicate scope for fruit load manipulation.

The current study corroborates the dominance of fruit sinks over vegetative growth for several degrees of severity of fruit load removal, applying the treatments to all fruit bearing nodes of the tree. The negative effect of fruit sinks on vegetative growth was also reported in other fruit tree species, e.g. olive (Haouari et al. 2012), apple (Meland 2009) and sweet cherry (Looney 1989; Whiting and Lang 2004). Regarding effects on leaf dry mass per unit leaf area, SLA, the current results are in agreement with the previous reports, e.g. Syvertsen et al. (2003) showing that fruit thinning increased SLA of ‘Spring’ navel orange trees (*Citrus sinensis* (L.) Osbeck), though the effects on SLA observed in both current experiments were relatively small. Still the tendency for lower SLA with higher fruit load is consistent with the idea that: with high fruit load resources are drawn from existing vegetative parts to fruits (Expt I) or less investment of dry matter per unit area of new leaves (Expt II).

Leaf photosynthesis, carbon and nitrogen

The current study confirmed that coffee, compared to many other tropical tree crops, has low maximum rates of net CO_2 assimilation, typically in the range of 4 - 11 µmol $m^{-2}s^{-1}$ (DaMatta 2003; DaMatta et al. 2007; Fahl et al. 1994) but comparable to other shade tolerant tree species (Poorter and Bongers 2006; Selaya and Anten 2010). Maximum photosynthetic rate per unit nitrogen per leaf area in coffee leaves is also relatively low compared to other C_3 species, e.g. potato (Vos and van der Putten 1998). For the same amount of nitrogen per unit leaf area, $A_{\max}$ of potato is ca. five times that of coffee.

In coffee, high fruit load, i.e. sink demand excess over source supply, has been associated with enhanced rates of leaf photosynthesis (Vaast et al. 2005) and conversely, source excess over limited sink demand (in case of low fruit load) with inhibition of photosynthesis by accumulating photoassimilates (Franck et al. 2006). DaMatta et al. (2008) found higher rates of leaf photosynthesis when fruit load/leaf area ratios were high supporting the notion that sink strength plays a role in driving photosynthesis rates. Still, these authors proposed that adaptation of leaf photosynthesis was independent of the relative strengths of sinks and sources, but rather directly related to effects on CO_2 availability in the leaf, resulting from changes in stomatal conductance. Compared to other treatments, stomatal conductance of T100 was relatively low (data not shown) but was in the range of g_s reported for coffee leaves (Araujo et al. 2008; Chaves et al. 2008; DaMatta et al. 2008). Araujo et al. (2008) found substantially lower g_m than g_s indicating that gm disproportionately limits A_{max} in coffee. In this study, diffusive limitation due to lower g_m seems likely for higher C_i in coffee trees with high fruit load.

There are indications that nitrogen may have been a limiting resource in the current experiments, implying N limitation is probably involved in the behaviour of the trees in this study. In contrast to previous studies in coffee (Vaast et al. 2005) and in olives (Haouari et al. 2012), leaf photosynthesis decreased rather than increased with increase in fruit load (Fig. 3a). In almond (Saa and Brown 2014) leaf photosynthesis also declined with increase in fruit load. In the current study, these lower rates of photosynthesis were associated with lower amount of leaf nitrogen per unit leaf area, (Na) (ANCOVA $p = 0.03$; Fig. 4) as the two are strongly correlated (e.g. Evans 1989). These results clearly demonstrate that the decrease in Amax associated with high fruit load cannot simply be associated with the straightforward feedback of sink demand or accumulation. Such feedback may occur when nitrogen is sufficiently applied to sustain high production.

Yield increased with fruit load in the first year (Figs. 6a and b), while nitrogen accumulation in fruits is approximately proportional to fruit yield. In addition to limiting carbon, limiting N probably contributed to reduced branch growth (Fig. 1) and appearance of fewer new leaves (Figs. 2b and d) and higher rate of loss of basal leaves of the branches (Figs. 2a and c) at high fruit loads than at low fruit loads. This view is reinforced by similar observations made in almond by Saa and Brown (2014). These authors found that fruit presence negatively affected leaf nitrogen content per unit leaf area and associated leaf photosynthetic capacity.

Source-sink regulation

Coffee trees tend to develop larger sink demand by fruits than source supply. However, this does not mean that the trees lack any mechanisms to adjust sink demand to resources. Fruit drop is such a mechanism; it may occur from the pinhead stage on during three months after blossoming (DaMatta et al. 2007). In the current experiments, there was consistent drop of fruits between the pinhead and harvesting stages (Fig. 5). Fruit drop greatly depends on fruit load and an increase in fruit drop with fruit load may indicate a shortage of carbon (and/or nitrogen) supply for fruit growth (Rivas et al. 2006). In the absence of fruit drop and if all fruits had grown to the same average dry weight then yields would be in proportion to fruit load. i.e. T25 initially only had 25% of the fruits compared to T100, but relative yields actually found in T25 were 55% and 60% of that of T100 plants in Expts I and II, respectively (Figs. 6a and b). The difference in ‘expected’ relative yield (25 %) and observed relative yield (ca 60 %) being due to larger weight per bean (22 % higher in T25 than in T100) and due to virtually no fruit drop in T25 against some 27 % in T100 (Fig. 5).

The adjustment of sink demand by variable fruit drop across treatments was not severe enough so as to prevent predisposition to low yield in the second year in trees with no (T100) or 25 % of the fruits removed (T75) in the first year. Essentially, there was crop failure in the second experimental year in T75 and T100 in both experiments. That low yield cannot be explained only from fewer new flower positions (nodes) in the T100 and T75 compared to T25 and T50 treatments. At the time of flowering in the second year (early February 2013) there were also fewer or no flowers observed per node in trees with high fruit load. So a direct effect of developing fruits or seeds on formation of flower buds seems likely; in apple such competition is seen as a primary cause for biennial bearing (Dennis and Neilsen 1999; Guitton et al. 2011). Further study would be needed to monitor accurately the dynamics of flower bud initiation and flower development.

Weight and size of beans

In the present study, we showed that decreasing fruit number per tree to 25 and 50% increased mean individual bean size and weight (Figs. 7a and b). Distribution over grade sizes was also changed (data not explicitly shown) resulting in larger fractions in the medium and large grades at the expense of the fraction of finer grades. Similar findings were documented for coffee in (Vaast et al. 2005; Vaast et al. 2006) and other fruit tree species, e.g. apple (Palmer et al. 1997) and peach (Corelli-Grappadelli and Coston 1991). The current study also revealed smaller relative variability the larger the mean bean dry weight (Figs. 7c and d). This indicates that the lower the fruit load more beans seem to be able to grow to their potential size.

Concluding remarks

Like in other fruit tree species (olive, apple and other horticultural corps), thinning coffee beans to low (25%) or medium (50%) fruit load enhanced vegetative growth and new branch development reducing competition for assimilate between fruit and vegetative organs. The practice improved bean size and weight; increased yield in the second year by stimulating new shoot extension (as in olive) and enhancing return bloom (as in apple).

This study provided new insight that the excess sink demand in high fruit load aggravated nitrogen limitation as reflected in lower leaf nitrogen concentration and associated lower A_{max}. In addition, this study further indicated that high fruit load in the first year, predisposed trees for a low yield in the next season mainly due to fewer new flowering nodes and presumably due to fewer flowers per node, perhaps via hormonal control as in apple (Dennis and Neilsen 1999). This leads to a pattern of biennial bearing. Manual reduction of fruits promotes vegetative growth and prevents biennial bearing. However, the articulation of clear guidelines, applicable in practice, needs further work.

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Chapter 5

Tree management and environmental conditions affect coffee bean quality through effects on resources for growth of beans

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Abstract

Coffees with specific taste and quality fetch higher prices. Although coffee plays a dominant role in the Ethiopian national economy, the country's coffee industry is generally characterized by low productivity and low quality. To address this issue, this study analysed the interactive effect of shade and nitrogen supply, fruit thinning and genotype by environment interactions on different coffee quality attributes. Organoleptic bean quality attributes declined with increase in radiation when nitrogen supply was limiting. In the absence of nitrogen limitation, however, the quality attributes hardly responded to radiation levels. In full sun, nitrogen had no effect on size and weight of coffee beans. Nitrogen supply improved bean size and weight at lower radiation levels. Fruit thinning and higher altitude significantly improved beverage quality, size and weight of coffee beans. Thinning beyond 50%, however, did not further improve the quality attributes. Effects of shade, nitrogen, fruit load and altitude are consistent with the proposition organoleptic quality and the size of coffee beans is promoted by factors and conditions that support non-limiting supply of resources for beans to grow and by a sufficiently long, temperature-dependent, period of maturation. Quality attributes did not differ much between varieties suggesting that crop management and growing environments might be more important than the genetic factors.

Keywords: caffeine, trigonelline, fruit thinning, non-limiting, organoleptic

Introduction

Coffee oversupply to the international market allows the market to select on quality (Belete et al. 2014). Coffees with good quality from specific origins and with specific taste (i.e. 'terroir coffees') fetch higher prices than bulk bean lots (Avelino et al. 2005; Läderach et al. 2011). Consumer demand for products differentiated on the inherent quality characteristics is also growing (Oberthür et al. 2011). So, in order to maintain one's share in the world market it is imperative to produce coffee of high and stable quality that meets consumer demand and preferences (Figueiredo et al. 2013; Subedi 2011).

Coffee plays a dominant role in the Ethiopian national economy (Davis et al. 2012) but, the country's coffee industry is generally characterized by low productivity (about 0.7 t ha^{-1} of dry green beans) and low quality (Alemayehu et al. 2008). Strengthening the position and price of Ethiopian coffee on the world market requires a systematic analysis of impacts of practices and procedures on coffee quality throughout the value chain, from farm to the point of delivery of green beans to the exporters.

The quality of coffee consists of (i) physical attributes: length, size or weight of coffee beans (ii) organoleptic characteristics: acidity, aroma, body, aftertaste, flavour, overall preference and balance and (iii) chemical constituents such as caffeine, trigonelline, chlorogenic acid (Agwanda et al. 2003; Behailu et al. 2008; Fox et al. 2013; Giomo et al. 2012; Joët et al. 2010; Lara-Estrada and Vaast 2007). Each of these attributes depends on factors such as shade level (Geromel et al. 2008; Lara-Estrada and Vaast 2007; Muschler 2001; Vaast et al. 2006), altitude (Avelino et al. 2005; Bertrand et al. 2006 ; Villarreal et al. 2009), daily temperature (Bertrand et al. 2012) and amount and distribution of rainfall (Silva et al. 2005). Physical and chemical characteristics of the soil (Behailu et al. 2008; Bosselmann et al. 2009), fertilizers (Lara-Estrada and Vaast 2007), genotype and provenance (Avelino et al. 2005; Belete et al. 2014; Bertrand et al. 2006 ; Leroy et al. 2006; Oberthür et al. 2011), agronomical measures like pruning and fruit thinning (Bertrand et al. 2006 ; Läderach et al. 2011; Vaast et al. 2006) were also reported to influence coffee quality. Previous studies concluded that shade improves physical and organoleptic attributes via lowering air temperature, which in turn slows down the ripening process leading to enhanced accumulation of aroma precursors (Bertrand et al. 2006 ; Joët et al. 2010; Lara-Estrada and Vaast 2007; Vaast et al. 2006). Fertilizer application improves bean size, weight and organoleptic quality through accumulation of fat and carbohydrates (Franca et al. 2005; Lara-

Estrada and Vaast 2007). In spite of all these efforts, quantitative understanding of the interactions between available nitrogen in the soil and level of radiation received by the coffee plant on coffee quality is still lacking. Effective quality management requires quantitative information on the effects of conditions, varieties and agronomic measures (Läderach et al. 2011).

Coffee berries act as priority sinks (Vaast et al. 2005) and can accumulate more than 95% of N, P and K absorbed by the plant during bean filling (Cannell 1985). Because of carbohydrate competition among berries it was reported that fruit over-bearing reduces bean size, weight (Vaast et al. 2005) and organoleptic quality of coffee beans (Bertrand et al. 2006 ; Cannell 1985). High fruit loads in 'year n' are likely to reduce flowering and bean production in 'year n+1' and gives rise to biennialy fluctuating yields (DaMatta 2004). In other species, e.g. sweet cherries (*Prunus avium*) (Whiting et al. 2005), kiwifruit (*Actinidia deliciosa*) (Smith et al. 1994), apple (*Malus domestica* Borkh) (Racskó 2006) proper management of crop loads (i.e. prevention of over bearing) was shown to enhance fruit quality. This indicates opportunity for crop load regulation to enhance coffee bean quality. The extent to which crop load management may similarly increase berry quality in coffee however is not well known.

Coffees from different regions and farms have specific quality and flavour (Avelino et al. 2005; Oberthür et al. 2011). Change in pest and disease pressure provides a motive to change to new varieties with adequate resistance against the biotic agents in question. The maintenance over time of the specific quality of a 'terroir' (Avelino et al. 2005; Boot 2005) requires insight in the degree to which these qualities are determined by the combination of climate and edaphic factors, prevalent at a site, on the one hand and the genotype on the other hand. However, few studies that we know of have attempted to quantify the extent to which variation in genotypes and environmental conditions contribute to specific quality of coffee.

Development of integrated and effective management packages along the coffee supply chain ensure stable production of high quality beans (Bertrand et al. 2008). Conducting dedicated experiments and identifying practices that determine quality along coffee supply chain, therefore, are important to inform actors in the chain on packages of conditions and management that contribute to quality (Läderach et al. 2011). In order to contribute to the development of such management packages, this study analyses (i) the effects of different levels of nitrogen supply and shading; (ii) fruit thinning and (iii) the magnitude of genotype x environment interactions on coffee physical and organoleptic quality attributes.

Materials and Methods

Expt - 1. Light, variety and nitrogen experiment

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) Horticulture farm, Jimma, Ethiopia (70° 33'N , 360°, 57' E and 1710 m a.s.l., data taken by GPS (GPS model 60, Garmin, Calgary Canada)). During the experimental period, the annual mean daily minimum and maximum temperatures of the site were 12.7 °C, and 28.2 °C, respectively and the annual mean minimum and maximum daily relative humidities were 49.4 % and 92.1 %, respectively (data measured with data logger, Log Tag, HAXO-8, China, mounted in the study area). The area receives an annual rainfall of 11300-1700 mm (Alemu et al. 2011).

Coffee (*Coffea arabica* L.) seedlings of varieties 74-4, 74-40 and 74-110 (Table 1) were raised in shaded nurseries from May 2011 for fourteen months before being transplanted to the experimental site where treatments were applied. Healthy and uniformly grown seedlings were selected and transplanted on 20 July 2012 to polybags of 60 x 60 x 60 cm dimension, filled with a mixture of subsoil and sand (3:1 v/v), arranged under four different artificial shade levels using neutral nylon screens (Moplaco Packaging private limited company, Addis Ababa, Ethiopia) installed horizontally at a vertical height of 2.30 m above the ground.

The study was conducted using a randomized split-split plot design with four levels of radiation: 100%, i.e. open sun without any shade, 70, 50 and 30 per cent of full sunlight as a main plot factor using shade nets abbreviated to R100, R70, R50 and R30, respectively. The main plots were split into three sub-plots to which the three coffee varieties were randomly assigned and each sub-plot was split into four sub-sub-plots to randomly accommodate four levels of nitrogen (0, 40, 64, and 88 g of N per plant per year, equivalent to 0, 150, 250 and 350 kg ha^{-1}), replicated three times. N treatments were abbreviated as N0, N40, N64 and N88, respectively. Each elementary plot consisted of six polybags (arranged in two rows of three) with one seedling per polybag. The spacing was in a grid of 1.6 m x 1.6 m between plants (i.e. a tree population density of 3906 ha^{-1}). Nitrogen, as urea, was applied in three split applications a year on 0, 116 and 236 DAT (days after transplanting) (i.e. 1-08-2012, 25-11-12 and 25-03-2013) in the first year and 374 and 487 DAT (10-08-2013 and 01-12-2013) in the second year and two times 681 and 798 (12-06-2013 and 07-10-2013) in the third year such that total applications corresponded with N treatments described above. Each tree also received 5 g P (63 kg ha^{-1} y^{-1} P) as Triple Super Phosphate (TSP) applied once a year, five days before N application on 26 July 2012 and 28 November 2013, following current recommendation (IAR, 1996). After application, both N and P fertilizers were thoroughly mixed into the soil to avoid losses from the soil surface. Based on the environmental conditions the seedlings were watered weekly, and hand-weeded as necessary. First yield of berries was harvested from 10 to 20 December 2014 (872 to 883 DAT). Growth and yield data were reported chapter 2 and 3, respectively.

Expt - 2. Fruit thinning experiment

The study was carried out in two consecutive years (2012/13 and 2013/14 production cycles) on Arabica coffee at two different sites. The first experiment (Expt 2a) was conducted from 7 June 2012 to November 2013 at 'Gomma-I' coffee plantation site located at 1570 m elevation, 7^o 57' N, 36^o 42' E, with annual mean daily minimum and maximum temperatures and rainfall of 14, 17°C and 1600 mm, respectively. The soil of the area is characterized as a Eutric Nitosol type, with pH ranging from 4.5 - 6.0 (Mekonnen and Belehu 2007). The second experiment (Expt 2b) was conducted from 14 May 2013 to November 2014 on a farmer's field located at Mana district of Jimma zone. The site is located at an elevation of 1750 m, 8^o 67' N, 37^o 07' E (GPS model 60, Garmin, Calgary Canada). The annual mean daily minimum and maximum temperatures and relative humidities of the area were, respectively, 14.2, 24.1 ^{o}C and 49.4 and 92.1 % (POWER 2012). The soil of the area was characterized as a Nitosol type, with pH ranging from 4.5 to 5.5.

In both cases, eight-year old Arabica coffee trees of cultivar 74-40 (resistant to coffee berry disease), were planted under shade trees (35 - 45% shade measured with a ceptometer, LP-80, Decagon Device, Inc., Pullman), at a spacing of 2.0 x 2.0 m between coffee plants and rows were selected and used for the experiments. Sixteen coffee trees of comparable size and morphology were selected for the study, taking care that the starting size of trees would not differ significantly among treatments. Following this selection, four fruit load treatments were manually imposed on all fruit bearing branches of the plants on 7 June 2012 (Expt 2a) and 14 May 2013 (Expt 2b). No fruits were removed in the control treatment while 25, 50 or 75 % of the fruits were removed from each node of the branches in the other three treatments resulting in four treatments namely T100, T75, T50 and T25. The experiments were arranged in a completely randomized block design with four replications. Data on yield and vegetative growth were reported in Bote and Vos (2016). Red berries were harvested from 18 to 26 December 2012 (Expt 2a) and from 28 October to 13 November 2013 (Expt 2b). Data on vegetative growth, yield as well as sizes and weights of individual beans are reported in (Bote and Jan).

Expt - 3. Genotype by environment interactions

The experiment was conducted at three locations, differing in altitude, in Manna district. Manna is one of the major coffee producing districts in Jimma zone with a total area of 47,898 ha of which 23% is low, 65% mid and the remaining 12% is high land (ARDO, 2008). Based on long-term (15 years) weather data obtained from the nearby meteorological station, the average annual rainfall, mean daily minimum and maximum temperatures of the district were 1523 mm, 13 ºC and 24.8 º C, respectively (ARDO, 2008). To monitor daily mean minimum and maximum temperature of the sites, data loggers were mounted on each site for three months at the beginning of the experiment. The collected data were then compared with data from NASA (POWER 2012) and FAO (FAO 2001) upon entering coordinates and elevation of the sites. Annual mean minimum and maximum temperature of the sites were then estimated using data from NASA as these correlated best ($R^2 = 0.89$) with the monitoring of our own. Mean daily temperatures calculated in this way were 17.1 ºC for the highest site (2063 m asl), 18.5 ºC for the mid altitude (1650 m asl and 21.1 ºC for the site at the lowest altitude (1410 m asl). The experiment comprised of eight coffee genotypes (Table 1). Fourteen months old plants were planted at each site in June 2012 in a randomized complete block design with three replications. Each experimental plot contained 25 plants with a spacing of 2 m x 2 m between plants and between blocks. Yield of berries was harvested from 21 October to 5 November, 29 October to 15 November 2014 and 20 December 2014 to 5 January 2015, from low, mid and high land, respectively. In the current analysis, data from only six varieties were used because at low land no sufficient yield was harvested from two varieties.

Measurements

In all experiments, only fully ripe, red coloured coffee berries were harvested from each individual tree (typically two to three rounds of picking). Fresh weight of berries at each harvest was recorded before processing and converted to dry clean beans (moisture content ca. 11%) with a conversion factor: 5.5 kg fresh berries = 1 kg clean, dry beans (Arcila - Pulgarín et al. 2002). Coffee fruit samples were then prepared in a wet processing method and dried according to standard agronomic practices to a moisture content of ca. 11%. Dry weights per bean (g) were obtained by weighing a subsample of 100 dry beans (Expts 1 & 3) and 1000 dry beans (Expt 2) per plot in three replicates. Bean diameters (mm) were determined by measuring width of 90 beans at their widest point and took the average of these 90 values for each treatment in each experiment using a digital calliper (Mitutoyo, IP 67, CD-20-PPX, Kawasaki, Japan). For cup quality analysis, 100 g green dried beans of each treatment within each experiment was roasted at 200°C for six minutes using a sample roasting machine (Probat, 4 Barrel Roaster, Germany) and ground to powder at the Ethiopian Commodity Exchange (ECX) laboratory, Jimma branch. Cup quality analysis was done by a panel of three cuppers on an infusion, prepared from 250 ml hot water (93°C) and 13.75 g of ground coffee. Cup quality was assessed using five criteria: aroma, body, acidity, flavour, aftertaste, balance and overall preference. Scoring was on an intensity scale of 6 – 10, with 0.25-point increments, following ECX testing protocol (ECX, 2010).

Table 1. Descriptions of coffee varieties used in the study

Expt	Variety	Green bean yield (kg/ha)		Growth habit	Year released	Growing Altitude (m asl)		
		On station	On farm			High*	Mid	Low
1	74-4	1660	800-900	open	1979/80	S	HS	S
1	74-40	1620	800-900	intermediate	1979/80	S	S	S
1	74-110	1910	900-1000	compact	1978/79	HS	S	Us
3	74-1	1220	600-700	open	1977/78	S	S	US
3	75-227[a]	1790	800-900	open	1980/81	HS	S	US
3	74-54	1830	800-900	intermediate	1980/81	S	S	S
3	74-112	1810	900-1000	compact	1978/79	HS	S	US
3	74-140	1970	900-1000	compact	1978/79	HS	HS	US
3	74-148	1800	600-700	compact	1979/80	HS	HS	US
3	74-158	1910	900-1000	compact	1978/79	HS	HS	US
3	74-165[a]	1730	800-900	compact	1978/79	HS	S	US

[a] Not used in the analysis because no sufficient yield was harvested from the two varieties, *high (1750 – 2100 m), mid (1550- 1750 m), low (1000-1550 m), S, suitable; HS, highly suitable; US, unsuitable (IAR 1996).

Data analysis

Data were analysed with Statistical Analysis System (v. 9.2, SAS Institute Inc., Cary, NC USA,) software. Data for Expt 1 were analysed as a split split plot design with radiation as the main plot, variety as sub plot and nitrogen as sub-sub plot. Data from Expt 2 were analysed separately for each year using a fixed-model one-way ANOVA (with fruit thinning considered fixed and blocks random) following a completely randomized block design. Data, Y, for Expt 3 were analysed using nested-factorial mixed-model ANOVA in which blocks, B, are nested within locations (with genotype, G, and locations, E, considered fixed and blocks random) as given in Eqn 1:

$$Y_{ijk} = \mu + G_i + E_j + (Ge)_{ij} + B(E)_{jk} + £_{ijk} \qquad \text{(Eqn 1)}$$

where:

Y_{ijk} is the k^{th} observation of the variable in the B^{th} block in Location **j** on genotype **i**; **μ** is the general mean; G_i and E_j represent the effects of the i^{th} genotype and the j^{th} location respectively. $(GE)_{ij}$ is the interaction effect between the genotypes and the location; $B(E)_{jk}$ is the effect of the k^{th} block within the j^{th} location, $£_{ijk}$ is the random error term associated with the kth observation on genotype i in location j due to uncontrolled factors. i = 6; j = 3; k = 3. The effects G_i\`, L_j, $(GL)_{ij}$ and $£_{ijk}$ are assumed independently and randomly distributed with zero means and equal variances. Significant differences between treatments means were separated using the Tukey's, honest significant difference, HSD test (P= 0.05).

Results

Expt - 1. Effect of radiation, nitrogen and varieties on coffee bean quality

Coffee cup quality attributes varied significantly among radiation as well as among nitrogen treatments (Table 2). Significant three way interactions (shade × variety × nitrogen) were observed for aroma, body and flavour. In the high radiation treatment, higher rates of N supply led to more aromatic beans in variety 74-110 and beans with heavier body in variety 74-4. Acidity, aftertaste, flavour, balance and overall preference, on the other hand, showed significant radiation (R) by nitrogen (N) interactions. In N limited coffee trees, the scores for these quality attributes declined with increase in radiation level (Figs. 1a - e). Radiation level had no significant effect on coffee beverage quality when ample N was supplied (N88). However, for flavour and aftertaste R30 had higher scores than any other radiation treatments (Figs. 1b and c). There was no significant main effect for varieties for any of the organoleptic attributes (Table 2).

Weight and size of coffee beans varied significantly among radiation as well as among nitrogen treatments (Table 2). There were also significant radiation (R) by nitrogen (N) interactions for the two variables. Higher rates of N supply led to heavier and larger beans

Table 2. Three factor analysis of variance of cup quality attributes, weight and size of coffee beans measured for three varieties, V, grown under different radiation levels, R (%) and rates of nitrogen supply, N (g tree^{-1}) (Expt 1).

Factors	Acidity	Aroma	Body	Aftertaste	Flavour	preference	Balance	BW^{a}	BS^{a}
R	6.58*	3.63ns	**5.05***	2.3ns	7.82*	2.12ns	**14.7****	**22.0*****	14.5**
V	0.84ns	1.39ns	3.11ns	0.38ns	1.2ns	2.49ns	2.16ns	0.57ns	0.0ns
R x V	0.99ns	0.19ns	0.82ns	2.48*	1.13ns	0.56ns	2.49*	1.96ns	1.63ns
N	**10.53****	**35.6*****	3.23*	**12.6*****	**24.39*****	**6.42*****	5.56*	13.2***	**19.5*****
R x N	2.76**	0.91ns	1.08ns	2.35*	5.85**	2.22*	3.27*	2.14*	2.17*
V x N	2.4*	0.12ns	2.65*	0.86ns	4.04**	0.55ns	0.69ns	1.18ns	1.13ns
RxVxN	1.46ns	1.74*	4.37***	1.1ns	4.38***	1.18ns	0.65ns	1.14ns	1.77ns

Numbers in the table are *F*- ratios of data pertaining to the first yield of berries in Expt 1, harvested from 10 to 20 December /2014 (872 – 883 days after transplanting, DAT). *F*-values of factors having the largest effects on the quality attributes are given in bold. ^{a}Bean weight (BW, g bean^{-1}); Bean size (BS, mm bean^{-1}). ns: $p > 0.05$; *: $P < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

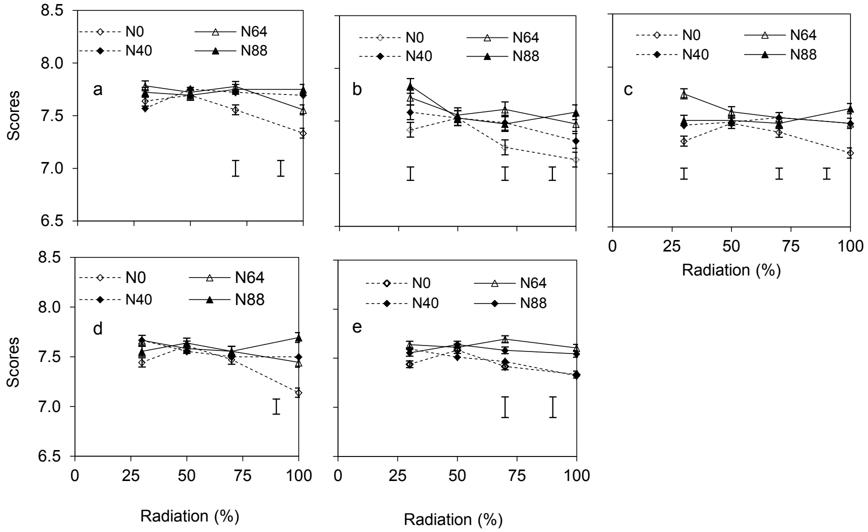

Fig. 1. (a) Acidity, (b) flavour, (c) aftertaste (d) balance and (e) the overall preference of coffee beans grown under different levels of radiation (R) and rates nitrogen supply (N). Data pertain to the first yield of berries, harvested from 10 to 20 December 2014 (872 - 883 DAT). Data points are means of nine values from three varieties with three replications. Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. The bottom bars represent LSD ($p < 0.05$) between treatments. Data were taken from Expt 1.

with no differences ($p > 0.05$) between N64 and N88 treatments. The magnitude of the N effect, however, declined with increase in radiation level resulting in the absence of significant N effects for R100. In R30, N supply (N88) increased mean bean weight and

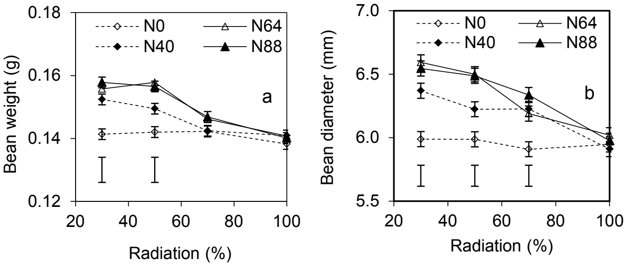

Fig. 2. (a) Mean weight and (b) diameter of individual coffee beans grown under different levels of radiation (R) and rates nitrogen supply (N). Data pertain to the first yield of berries, harvested from 10 to 20 December /2014 (872 - 883 DAT). Data points are means of the average of nine means (sample size 100 and 30 beans respectively for bean weight and bean diameter) averaged across three varieties with three replications. Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. The bottom bars represent LSD ($p < 0.05$) between treatments. Data were taken from Expt 1.

individual bean diameter, respectively by 12 and 9.8% (Figs. 2a and b). Radiation had no effect on weight and size of beans grown without application of fertilizer N (N0 treatments).

Expt - 2. Effect of fruit thinning on coffee bean quality

Fruit thinning enhanced coffee bean beverage quality (Table 3). In both Expts 2a and 2b, the trend indicated higher acidity, aroma, body, aftertaste, flavour, balance and overall preference the lower the fruit load with an optimum curve, peaking at T50. Thinning beyond 50%, did not further improve coffee beans physical and beverage qualities. Fruit thinning did not significantly affect coffee bean balance. Similar to shade and nitrogen nutrition, fruit thinning significantly improved individual weight and size of coffee beans (Bote and Jan 2016). Compared to beans in T100, dry weight and individual bean size in T25 increased by an average of 20 and 13 %, respectively.

Expt - 3. Genotype by environment interactions

Higher altitude significantly enhanced coffee beverage quality, weight and size of individual beans (Table 4). Though there were significant genotype × environment interactions (Table 4), the higher value of the *F*- ratio for environments compared to that of variety and genotype × environment showed that environmental differences accounted for the major part of total variation in most of the variables assessed (from 46 % for balance to 60% for body). Averaging across varieties, coffees grown in high altitude (mean annual temperature 17.1 ºC) had significantly higher body, balance and flavour scores than coffees grown in low (21.2 ºC) and in mid (18.5 ºC) altitudes (Figs. 3a and b; 4a and b). Similar trends were observed for acidity and overall preference. These trends were similar for most of the coffee varieties, but difference in acidity between mid and high (Fig. 3a) and differences in aroma and overall preference between low and mid (Figs. 3a and b, Table 5) were not statistically significant. Coffee beans from the high altitude site were also more aromatic than beans from low- and mid- altitude sites (Fig. 3a, Table 5).

Across varieties, individual weight and size of coffee beans increased with altitude (Table 5). Mean individual dry weight of coffee beans at the highest was 24 % heavier than beans at the lowest altitude. Similarly, mean individual size (diameter) of coffee beans grown at high land was 3 to 7 % greater than for low land (Table 5).

Table 3. Means of bean quality attributes of field grown coffee trees as affected by different fruit load treatments.

Expt	Fruit load %)	Acidity	Aroma	Body	Aftertaste	Flavour	preference	Balance
2a	25	7.67^{ab}	7.5^{a}	7.58	7.25^{b}	7.75^{a}	7.58^{ab}	7.67
	50	7.92^{a}	7.5^{a}	7.75	7.58^{a}	7.92^{a}	7.83^{a}	7.75
	75	7.50^{b}	7.1^{b}	7.42	7.17^{b}	7.33^{b}	7.42^{bc}	7.42
	100	6.91^{c}	7.2^{b}	7.00	6.99^{c}	7.10^{c}	7.01^{c}	7.08
	P	**0.04**	**0.04**	**0.21**	**0.04**	**0.02**	**0.02**	**0.06**
2b	25	7.77	7.35^{b}	7.67^{a}	7.17^{b}	7.25^{b}	7.25^{b}	7.37
	50	7.73	7.50^{a}	7.69^{a}	7.60^{a}	7.69^{a}	7.71^{a}	7.65
	75	7.38	7.49^{a}	7.33^{b}	7.53^{a}	7.59^{a}	7.60^{a}	7.55
	100	7.28	6.95^{c}	7.25^{b}	6.93^{c}	7.11^{b}	7.17^{b}	7.11
	P	**0.07**	**0.013**	**0.02**	**0.01**	**0.01**	**0.01**	**0.25**

Expt 1 conducted in 2012/13 and Expt 2 conducted in 2013/14 at different production years and sites. The scores for each of the quality attributes were based on a scale of 6 - 10 with 0.25 increments (6 = lowest, 10 = outstanding) (ECX, 2010). Means followed by same letter in the same column were not statistically significantly different from each other according to Tukey, HSD test (5%).

Table 4. Interaction effects of genotype (G) and environment (E, location) on cup quality attributes, bean weight, BW (g bean^{-1}) and bean size, BS (mm bean^{-1}) of coffee beans.

Factor	Acidity	Aroma	Body	Aftertaste	Flavour	preference	Balance	BW^{a}	BS
E	8.9^{**}	5.33^{*}	30.3^{***}	1.24ns	10.5^{**}	11.1^{**}	23.1^{***}	47.4^{***}	5.1^{*}
G	2.87^{*}	1.87ns	2.62^{*}	0.42ns	1.47ns	2.72^{*}	4.96^{**}	17.1^{***}	3.3^{*}
E*G	2.28^{*}	2.45^{*}	2.67^{*}	1.6ns	3.6^{**}	2.13^{*}	2.9^{*}	2.3^{*}	2.6^{*}

Numbers in the table are *F*- ratios. *F*-ratio of factors having the largest effect on the quality attributes are given in bold. ns: $p > 0.05$; *:$p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Table 5. Means of bean quality attributes of coffee beans as affected by genotype and environment and their interaction.

Location	Variety	Acidity*	Aroma	Body	Flavour	Balance	Preference	BW	BS
Low	74-1	7.5^{d}	7.75^{cd}	7.67^{d}	7.5^{e}	7.42^{e}	**7.75^{d}**	13.80^{ef}	6.19^{c}
	74-54	7.30^{e}	7.5^{e}	7.67^{d}	7.5^{e}	7.67^{d}	**7.67^{de}**	15.48^{d}	6.24^{c}
	74-112	7.25^{e}	7.58^{de}	7.0^{f}	7.42^{ef}	7.62^{d}	7.25^{e}	11.92^{g}	6.15^{c}
	74-140	7.83^{c}	7.50^{e}	7.08^{f}	7.58^{de}	7.67^{d}	7.25^{e}	12.70^{fg}	6.16^{c}
	74-148	7.82^{c}	**7.83^{c}**	7.25^{ef}	**7.83^{c}**	**7.65^{d}**	7.08^{f}	13.44^{f}	6.31^{bc}
	74-158	**8.0^{c}**	7.42^{e}	**7.83^{c}**	7.33^{f}	**7.67^{d}**	7.08^{f}	13.75^{ef}	6.29^{bc}
Mid	74-1	8.17^{b}	7.58^{de}	7.83^{c}	7.58^{de}	7.25^{f}	7.83^{c}	16.21^{d}	6.3^{bc}
	74-54	7.83^{c}	7.58^{de}	7.33^{e}	7.75^{cd}	7.92^{b}	**8.17^{a}**	16.15^{d}	6.45^{ab}
	74-112	7.42^{de}	**7.83^{c}**	7.75^{cd}	**8.08^{b}**	**8.00^{b}**	7.75^{d}	13.93^{ef}	6.22^{c}
	74-140	**8.40^{a}**	7.67^{d}	**7.83^{c}**	7.83^{c}	7.50^{e}	7.83^{c}	12.93^{f}	6.48^{ab}
	74-148	7.92^{c}	7.65^{d}	7.75^{cd}	7.50^{cd}	**8.00^{b}**	7.67^{de}	14.38^{e}	6.38^{bc}
	74-158	**8.33^{ab}**	7.67^{d}	**7.83^{c}**	7.58^{de}	7.83^{c}	7.75^{d}	17.35^{c}	6.19^{c}
High	74-1	7.83^{c}	8.08^{b}	8.25^{b}	8.25^{a}	**8.33^{a}**	**8.17^{a}**	18.33^{b}	6.39^{b}
	74-54	8.17^{b}	**8.25^{a}**	8.25^{b}	**8.25^{a}**	8.25^{a}	7.92^{bc}	18.96^{a}	6.42^{b}
	74-112	**8.42^{a}**	7.58^{de}	7.92^{c}	7.58^{de}	7.83^{c}	7.58^{e}	15.87^{d}	6.36^{bc}
	74-140	7.83^{c}	7.58^{de}	7.92^{c}	7.67^{d}	7.75^{cd}	**8.03^{b}**	15.63^{d}	6.47^{ab}
	74-148	7.82^{c}	7.83^{c}	**8.58^{a}**	8.17^{ab}	7.92^{b}	**8.01^{b}**	14.42^{e}	6.39^{bc}
	74-158	**8.45^{a}**	7.67^{d}	8.08^{b}	7.92^{c}	7.83^{c}	**8.17^{a}**	17.49^{bc}	6.56^{a}

BW, bean weight; BS, bean size; *the scores for each of the quality attributes were based on a scale of 6 - 10 with 0.25 increments (ECX, 2010). Means followed by same letter in the same column across all locations did not differ significantly from each other according to Tukey, honestly significant difference test (5%). Highest values at each location are indicated in bold while the lowest values are underlined.

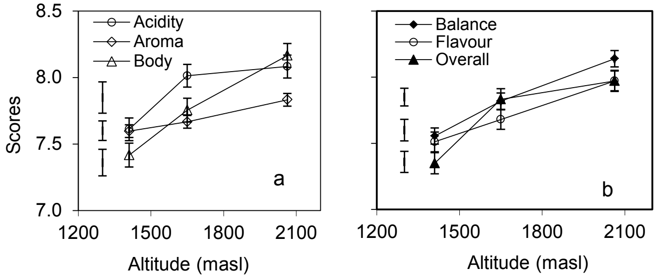

Fig. 3. (a) Acidity, body and aroma, and (b) overall preference, flavour and balance of coffee beans as affected by the altitude. Data points are means of 18 values averaged across six varieties and three replications. Vertical bars indicate mean ± 1SE. When not shown, SE was smaller than the symbol. The side vertical bars represent LSD ($\boldsymbol{P} < 0.05$) for (a) acidity, aroma and body; (b) balance, flavour and overall, respectively from top to bottom.

Discussion

This study analyzed coffee quality in relation to: (i) different shade and nitrogen levels using three different coffee varieties, (ii) tree management (fruit thinning) and (iii) examined the extent to which genotype and the growing environment determine the organoleptic and bean physical quality. The study is one of the first to show the interaction effects of shade and nitrogen supply on coffee bean quality. Both shading and nitrogen supply significantly influenced coffee bean qualities, but there was an interaction between the level of shading and nitrogen supply: organoleptic bean quality attributes generally declined with radiation when N supply was limiting, but these quality attributes responded little and unsystematically to radiation level in the absence of nitrogen limitation (Table 2, Fig. 1). For weight and size of beans, the opposite was true: in N0 treatments, the same low values were found irrespective of radiation level. In full sun (R100) there was no effect of N on bean weight and bean size, but when N was applied both attributes became larger the lower the radiation level and the larger the rate of N supply (but no differences between N64 and N88 treatments). This is mainly due to the distinct effects of radiation and nitrogen on size and flavor of coffee beans.

There is a widespread perception of the better coffee quality from trees grown in shade as compared to open sun cultivation (e.g. Geromel et al. 2008; Muschler 2001; Vaast et al. 2006). This proposition is not unconditionally true (Bosselmann et al. 2009). The alternative proposition that we want to advance is: organoleptic quality of coffee beans is promoted by factors and conditions that support non-limiting supply of resources for beans to growth and by a sufficiently long, temperature-dependent, period of maturation.

A postulate of that general proposition is that the substrate supply per bean is better under shade than under full sun. This can be the case if the source to sink ratio is higher under shade than in full sun. Compared to full sun, shade can promote source to sink ratio when fewer berries per tree develop, even fewer than in proportion to the decline in substrate production associated with the reduction in radiation receipt under shade. Such a situation would enhance substrate supply per bean and reduce competition among the developing beans, allowing the beans to realize their full growing potential (Cannell 1985; Vaast et al. 2005).

Muschler (2001) examining the effect of shade on quality in a marginal (low, warm) environment hypothesized that shade promotes slower and more balanced filling and uniform ripening of berries, thus yielding a better-quality product than unshaded coffee plants. Shade promotes quality if shade masks or counteracts a limitation that is manifest under full sun. Our findings in Exp. 1 supports this proposition: when N was limiting organoleptic attributes tended to decline with exposure to higher radiation level, but these attributes did not respond to radiation when ample N was supplied (Fig. 2).

Apart from changes in source to sink ratio, shade can also reduce canopy and fruit temperature as compared to exposure to full sun (Geromel et al. 2008; Guyot et al. 1996; Muschler 2001; Vaast et al. 2006). DaMatta et al. (2007) mentioned 2 – 4 ^{o}C lower temperature in shade than in sun. Temperature responses of developmental processes such as the growth and maturation of coffee beans often show an optimum curve. In the suboptimal temperature range for rate of development reduction in temperature prolongs the period of filling of the bean, so there is more time to accumulate constituents. Lower temperature by shade only is positive if shade compensates for or masks the negative effect of another factor (e. g. nitrogen limitation) on coffee bean quality.

Altitude is also a strong determinant of quality (Avelino et al. 2005; Bertrand et al. 2006 ; Decazy et al. 2003; Joët et al. 2010; Lara-Estrada and Vaast 2007). In high altitude environments, which are relatively cool, bean filling takes already a relatively long time (Bertrand et al. 2006 ; Geromel et al. 2008; Vaast et al. 2006). Shade and associated lowering of temperature may not improve the growth potential and quality of beans growing under those conditions, whereas shade and reduction of temperature may show positive effects in low, warm environments. Such mechanism may explain why Bosselmann et al. (2009) observed that the effect of shade depended on altitude. These authors reported negative effects of shade on components of quality at high altitudes.

Nitrogen supply stimulated tree performance and enhanced vegetative vigor of coffee trees via improving their RUE (Chapter 2). Trees with higher vegetative vigor supply more assimilates to berries (Bertrand et al. 2006 ; Leroy et al. 2006). The current study (Figs. 1 and 2) corroborates that adequate fertilization promotes organoleptic quality as well as bean size (Lara-Estrada and Vaast 2007), this is most likely through enhanced production of substrates for the growth of beans. The positive effects of fruit load reduction on quality (Table 3) are also consistent with the proposition that source to sink ratio is a strong determinant of quality. The study further corroborates previous observations that fruit thinning improves beverage quality, size and weight of coffee beans (Bertrand et al. 2006 ; Cannell 1985; Vaast et al. 2005; Vaast et al. 2006). Apparently, at 50 % fruit load the maximum organoleptic quality had been reached with no further improvement when fruit load was reduced to 25 % (Table 3). For countries like Ethiopia, where ordinary labour is cheap, fruit thinning may be an economically viable management practice since the premium paid for high coffee quality can amount to more than 100% of the market price of ordinary coffee quality

Factors and conditions promoting organoleptic quality generally also promote bean growth. There seems to be some association between organoleptic quality attributes and weight and size of beans. This is because ample availability of resources for growth, hypothesized to enhance organoleptic quality, is also promoting dry matter accumulation in beans. Higher organoleptic quality of bigger beans explains the price bonus for the larger grades, smaller beans of the same variety bring lower prices (Leroy et al. 2006). However, in Expt 1, there was no association between quality and weight and size of beans in treatments with N fertilization: the decline in weight and size of beans with increase in radiation level (Fig. 2) was not associated change in quality attributes (Fig. 1). In the N0 treatments bean weight and diameter did not respond to radiation but organoletic attributes tended to decline with radiation level.

Differences in coffee bean quality attributes among varieties were only of marginal significance in the current study. This is in agreement with the results documented in Bertrand et al. (2006) and Moreno et al. (1995). Bertrand et al. (2006) compared compositions and beverage qualities of Arabica hybrids involving Sudanese-Ethiopian origins with traditional cultivars in three countries (El Salvador, Costa Rica and Honduras) and found no significant differences among varieties. Moreno et al. (1995) evaluated physical and cup quality attributes of Colombia, Typica, Bourbon and Caturra coffee varieties and reported that all varieties produced beans of similar quality. The marked differences in bean physical and organoleptic qualities observed among shade levels, nitrogen supply, fruit thinning and the growing environments appeared as a general trend in most of the varieties indicating the importance of crop management and growing environment relative to the genetic factors. This corroborates the statement that sub-standard crop managements practices are the actual factors behind poor coffee bean quality rather than inbreeding with *C. canephora* with the aim of improving disease resistance of *C. arabica* (Van der Vossen 2009).

Table 6 presents an overview of the differences in the mean of the organoleptic quality scores, comparing within experiments the treatment or condition yielding the highest and the one yielding the lowest mean score. Though several interactions between factors are not accounted for, these data indicate the improvement in quality that can be achieved at most when moving from the worst condition or treatment to the best condition or treatment.

Table 6. Compilation of maximum effects of treatments and condition on organoleptic quality.

Management factor	Treatments compared	Range in mean quality score	Gain in quality score
Full sun to shade limiting N	R100 N0 and R30N0	7.30 to 7.42[*]	0.12
Full sun to shade limiting N	R100 N0 and R50 N0	7.30 to 7.55	0.25
Full sun to shade non-limiting N	R100 N88 and R30 N88	7.68 to 7.65	0.03
Nitrogen under shade	R30 N0 and R30 N88	7.42 to 7.65	0.23
Nitrogen in full sun	R100 N0 and R100 N88	7.30 to 7.68	0.38
Altitude and variety	difference between varieties at low altitude	7.39 to 7.65	0.26
	difference between varieties at high altitude	7.85 to 8.17	0.32
Altitude difference	Difference between low and high altitudes	7.57 to 8.01	0.44
Fruit thinning	T50 and T100 (average of Expts)	7.08 to 7.69	0.61

[*] Numbers are the means of scores on the average organoleptic quality attributes: acidity, aroma, body, aftertaste, flavour, overall preference and balance.

The first two lines in Table 6 indicate that comparatively speaking; the negative effect on quality of exposure to full sunlight is limited. Larger decline when comparing R50 and R100 (-0.25 points) than when comparing R30 and R100 (-0.12 points) are explained by the general tendency for quality attributes to be higher in R50 than in R30 (Fig. 1). In the absence of nitrogen limitation, there is no effect of radiation regime on the mean score of quality attributes. Within radiation regimes, removing nitrogen limitation improves the mean by 0.23 points in shade and with 0.38 points in full sun.

Within varieties comparison across altitudes shows a gain of 0.39 – 0.55 points with increasing altitude from 1500 to 2100 m asl depending on the variety, implying that the effect of a 600 m increase in altitude can be larger than the effect of change in radiation regime or an increase from 0 to 88 g tree^{-1} in nitrogen supply. Within altitudes and across varieties the improvement due to variety choice was maximally 0.26 points at low altitudes and 0.32 points at high altitude, confirming the modest effect of variety choice. Of all factors investigated, however, reducing fruit load to 50 % gave the highest improvement in quality score (0.61 points).The compilation of treatment effects on quality provides a basis for practical decision-making. Overall, the study indicated that nitrogen application, shading and control of fruit can all significantly contribute to bean quality.

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Chapter 6

General discussion

General discussion

Need for efficient production of higher yield and constant high quality

Intensive agriculture spares land for nature because it is often assumed that for a fixed demand, higher yields decrease the area that needs to be cultivated (Lambin and Meyfroidt 2010). Improved agronomic practices e.g. optimized fertilization, integrated pest management, control of soil erosion and weeds and effective shade management are, however, likely to be more profitable, boost yields (e.g. coffee yield by 20 to 30%, Kwan and Cervone (2014)) and improve product quality.

Crop yields are part of a range of ecological, social and economic benefits delivered by farming systems. When land is limited, high yields per unit area become central to sustainable food security (Godfray et al. 2010). Intensive production of cash crops such as coffee can contribute to local food security if the cash earned by selling the commodity from a given piece of land earns farmers enough money to buy more food than they could produce on the same pieces of land. In Ethiopia for example, coffee exports and sales greatly contribute to food security and to the national economy, ca. 33% (Davis et al. 2012). Producing more yields from the same area of land while reducing the environmental impacts, however, requires what has been called “sustainable intensification” (Crowder et al. 2010). A central challenge for sustainability is how to preserve forest ecosystems and the services that they provide us while enhancing crop production (Lambin and Meyfroidt 2011).

Coffee is the world’s most important cash crop but its cultivation is changing from traditional agroecosystems to management systems where shade trees are removed and N fertilizer is applied at high rates even up to ca. 800 kg N ha^{-1} yr^{-1} (Bruno et al. 2011). The practice increases coffee bean productivity and its supply to the world market. Consequently, potential for substantial loss of N to groundwater and the atmosphere is also increased. Babbar and Zak (1995) for example, reported a threefold (24 kg ha^{-1} y^{-1}) annual leaching losses of NO_3^- from unshaded plantations compared to coffee trees grown in shaded plantations (9 kg ha^{-1} y^{-1}).

The worldwide coffee surplus along with the changing patterns in global coffee commodity chains (e.g. abolishment of quota systems), on the other hand, has caused coffee prices to decline to their lowest levels in a century (Castro-Tanzi et al. 2012). Increased global production also resulted in a fundamental change at the consumption side; the customers became more interested to drink a better quality coffee (Alvarez et al. 2010), raising the needs for production of coffee with specific taste and better quality.

Coffee is the backbone of the Ethiopian economy (Davis et al. 2012) but the country's coffee sector is generally characterized by low productivity and low bean quality (Alemayehu et al. 2008; Labouisse et al. 2008). This reduced the competitiveness of the country in the world market and raised questions about the sector's long-term viability. Ethiopia's coffee farmers face difficulty to sustain their lives and to send their children to schools. Improving Ethiopian coffee productivity and quality with the ambition of remaining competitive in the world market needs a public-private consortium and coordinated effort on the part of stakeholders at different stages of the supply chain. New approaches and options could complement the current production systems and the overall efficiency of Ethiopian coffee production.

Thesis approach

In this thesis, we looked at manageable factors and measures to explore options for improving Ethiopian coffee productivity and quality. Exploring these possible options, however, requires a good understanding of the morphological and physiological responses of coffee trees to the changes effected by these management practices and how these responses in turn determine growth and productivity of the trees. To better understand coffee tree responses and underpin rational tree and system management, we analysed vegetative growth, biomass production and radiation use efficiency (Chapter 2), nitrogen uptake, N distribution, leaf photosynthesis and bean yield (Chapter 3) of coffee trees grown under different radiation levels and nitrogen fertilizers rates. We further explored how coffee trees responded to manual fruit load manipulation and evaluated morphological, physiological and yield responses of coffee trees to the practice (Chapter 4). In Chapter 5 of the thesis, we reported responses of coffee quality attributes to radiation and nitrogen, fruit load manipulation and genotype by environment interactions. In this chapter (Chapter 6), the main findings with regard to the research questions are summarized and general conclusions based on the findings of the studies presented in the previous chapters are described. The chapter concludes with the overall contribution of the thesis and possible recommendations with future research direction.

Main findings

Vegetative growth and biomass production

Nitrogen is a major mineral nutrient for plants and plays a central role in biomass production of all plants (Bruno et al. 2011). Adequate supply of nitrogen fertilizer is essential for the expansion and photosynthetic functioning of plant canopies (Grindlay 1997). In current intensive systems of coffee cultivation in open sun, more N is needed to sustain coffee bean quality and productivity. In this context, we tested the hypothesis that gains in bean quality and productivity can be made if management of shade and nutrients are based on more profound insight in dependency of nitrogen on the amount of available radiation. We also answered the research question 'how do coffee trees respond to different radiation and nitrogen supply rates in terms of morphological and physiological responses' stated in the introduction.

In this study, we showed that more biomass was produced the higher the radiation level when larger N was supplied (Chapter 2). Variations in biomass production in response to N supply depend on incident radiation, IR (MJ d^{-1}), fraction of intercepted radiation by the canopy, *FI,* and radiation use efficiency, RUE (g MJ^{-1}). *FI* in turn depends on the leaf area index, LAI, and *k*, the radiation extinction coefficient. LAI is the main physiological determinant of crop yield and is primarily determined by quantity and size of individual leaves. Leaf number and branch number increased with increase in the level of nitrogen supply (Chapter 2). N supply contributed to more leaf size expansion and increased LAI is a consequence of higher growth rate of leaf area. With increasing LAI, the fraction of light absorbed and cumulative intercepted radiation per plant increased, hence higher biomass production per tree. In response to radiation, however, leaf size decreased (Chapter 2). This may seem to negatively affect biomass production per tree. The larger number of leaves per tree at higher the radiation level, however, counteracted this effect.

The relative differences in biomass production among coffee trees grown in open sun and shaded environment were related to differences in radiation use efficiency, RUE. Both photosynthetic rates and N content per unit leaf area affected plant's RUE. We showed that sun plants had higher photosynthetic rate and higher leaf nitrogen contents per unit leaf area (N_a) than shaded plants (Chapter 2). In this context, we showed also that RUE of coffee trees increased with increasing N supply with a larger effect observed in high (86%) than in low (40%) radiation level (Chapter 2). So far, relatively little research has been done to understand the mechanisms that underlie coffee growth responses to variation in light availability and how this is mediated by N supply. This study brought new insight that the biomass advantage in open sun is explained by the increase in RUE due to increase in N supply. This increase in RUE values is, however, to the detriment of light interception. Compared to trees in shaded environment, trees in high radiation environment had leaves with smaller leaf area in such a way that they capture less light but utilize it more efficiently. Previous work (e.g. Vos and van der Putten 1998) indicated similar adaptation strategy in potato (*Solanum tuberosum* L.).

This study also measured for the first time the light extinction coefficient, $k = 0.44$ for coffee trees. This parameter describes the distribution of light within the crown of a tree and is thus an important input variable for coffee growth models. The estimated k value is in the range of values (0.3 – 0.8) reported for most plant communities (Monsi and Saeki 2005). Plants grown at high light exhibited lower coefficient values than those growing in shade (data not shown) and this is in line with the hypothesis that a small extinction coefficient is advantageous for biomass production under stronger radiation while a larger one is more advantageous under weaker radiation (Kitajima et al. 2005). The decrease in k value the higher the radiation is an indication of the relative vertical inclination of sun leaves compared to shade leaves orientation (Monsi and Saeki 2005). This pattern allows uniform light distribution among coffee leaves in open sun and enhances mean photosynthetic rate per unit ground area of the canopy as a whole (Kitajima et al. 2005).

Morphological modifications such as increase in plant height and specific leaf area could contribute to the increase in tree growth as well (Grime 1994; Poorter 1999). In this study, shade grown trees had greater plant height and larger specific leaf area, SLA, than those grown in full radiation (Chapter 2). Such morphological responses were also observed in other plant species ranging from grasses (Gubsch et al. 2011) to legumes (Roscher et al. 2011) and to perennial trees like conifers (Claveau et al. 2002). Taller stature enables plants to overtop neighbour plants and thus prevent having to grow in the shade. This response has been associated with the so-called shade avoidance syndrome and was notably stronger at high N than at low N supply. At high N availability competition for light might be relatively more important and thus under these circumstances strong shade avoidance might be more beneficial.

In general, interspecific variation in relative growth rates tends to be strongly and positively correlated with variation in SLA (Knops and Reinhart 2000). SLA consists of two components: leaf thickness and leaf mass density i.e., leaf dry mass per unit green leaf volume

(Witkowski and Lamont 1991). In this study, SLA shifted along the radiation gradient towards more but thinner leaves the lower the radiation level. The result is also among the most conspicuous results of this study. Larger SLA increases the competitive advantage of the coffee trees for the available radiation under the canopy of the shade trees. For the light competition, trees allocated relatively more biomass to the aboveground organs (high leaf weight ratio, LWR; high leaf area ratio, LAR) than to the belowground organs (low root shoot ratio, RSR) compared to trees grown in open sun (Chapter 2).

We showed higher light saturation of leaf photosynthesis rate, A_{max}, with more radiation and more nitrogen (Chapter 3). The result was consistent with previous reports (e.g. Anten et al. 1996; Fahl et al. 1994). Most of these differences in A_{max} are associated with effect of both radiation and nitrogen supply on leaf nitrogen content per unit leaf area, N_a (g m^{-2}). Plants grown under high radiation environments with larger nitrogen supply, exhibit high N_a and thus high A_{max} (Chapters 2 and 3). The positive effect of N availability on photosynthetic capacity ensures high photosynthesis at high radiation and simultaneously helps avoid photo damage (Fahl et al. 1994; Ramalho et al. 1999; Ramalho et al. 2000). The latter, because more light energy is used for photosynthesis and because more N is available for other light absorbing compounds. Conversely, the higher SLA found for shade tree may confer advantages in shade as it allows for efficient light capture per unit mass.

Uptake and distribution of N in vegetative plants and bean yield response

The ability of a crop to convert soil nitrogen into biomass depends on the crop's N-uptake and utilization. In this study, we showed positive interactions of radiation and nitrogen supply on both N-uptake and biomass production (Chapter 3). At high radiation, more N was taken up and more efficiently utilized. Larger radiation interception per plant enlarges the carbon resources for growth of the root system and enhances the requirement for N. Notably the apparent nitrogen recoveries, ANR (i.e. the slope of the response of N uptake to N applied), that we found for coffee trees were relatively low compared to the values reported for other crops, e.g. potato (Vos and Van der Putten 2001), but were in agreement with typical values obtained in other coffee studies (e.g. Fenilli et al. (2007); (2008); Cannavo et al. (2013)); as well as values reported for other tree crops (e.g. young pear (Neto et al. 2006) and peach (Nario et al. 2003)). The continued response of coffee trees to increase in N supply, coupled with low ANR, is probably the main reason why high N rates (e.g. 800 kg ha^{-1} y^{-1}, see Bruno et al. (2011)) are used in open sun coffee productions.

In this thesis, we showed that total nitrogen concentration in the whole plant (mg g^{-1}) increased with N supply and was higher for plants grown in low than in high radiation environment. In leaves, N_a (g m^{-2}), increased with increasing nitrogen supply but was lower for plants grown in lower radiations (Chapter 3). The lower N_a for plants in low radiation environment was associated with increases in SLA. In shaded trees, less nitrogen is allocated to soluble proteins and nitrogen in pigment protein complexes increases. The pigment-protein complexes spread over a greater area (higher SLA) and capture more light (Evans and Poorter 2001).

Optimization of fertilizer application

In the thesis (Chapter 3), we indicated that both shading and nitrogen limitations reduced coffee bean yield. We also showed that yield is positively and strongly associated with tree biomass (Chapter 3). That is, traits (e.g. RUE) underlying biomass production are equally important for yield. We assumed farmers would get 1.65 USD per kg dry green beans and pay 1.09 USD per kg N (Girmay 2015). Based on these numbers and the second order polynomial response of bean yield to N input (Chapter 3, Fig. 7b), we calculated the cost benefit relation of fertilizer supply at farm gate level and calculated that an average of 76 g nitrogen per tree per year (equivalent to 296 kg ha^{-1} y^{-1}) produces the highest net benefits for Ethiopian coffee farmers. This optimum was not very sensitive to the radiation level and pretty well matched with the economically optimum N input level of 300 kg ha^{-1} y^{-1} that we derived from data collected from field-grown coffee trees by Endris et al. (2008). Coffee trees ANR is low. Recommending such quantities of N input, even if economically justifiable, would require insight in the fate of N not taken up. Good estimates of leaching losses and N_2O production are generally needed to arrive at ecologically justifiable levels of N input. The current results on nitrogen responses of coffee are also relevant to organic coffee producers. Estimates of mineralization allow assessing the degree of N limitation in organic fertilizer (e.g. manure, compost).

Fruit load manipulation

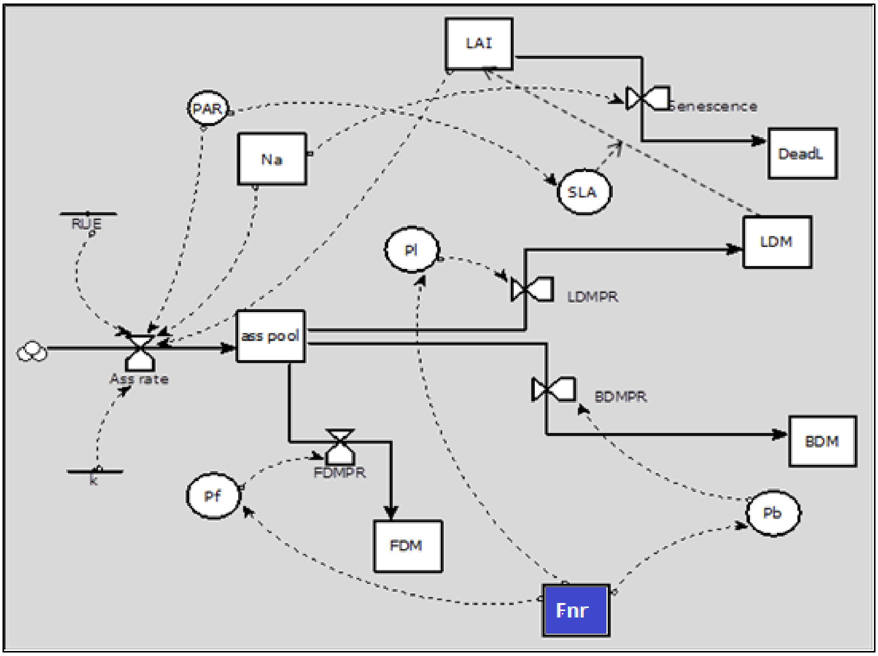
High fruit load results in high resource demands for the growth of those fruits, which may go at the expense of resource availability for vegetative growth. In this study, we showed the relative strength of fruit sinks over vegetative growth, unravelled the dynamics of mechanisms causing bienniality in coffee and its consequences for competition for carbon and nitrogen. Removing fruits at a very young stage stimulated branch growth and new leaf production while leaf shedding was reduced (Chapter 4). In coffee, new branches and extension of the existing ones are essential as they provide new flower positions that can develop into fruits in the next season. Part of the mechanism behind the biennial pattern in coffee is thus as follows. High fruit load in one year reduces branch growth through resource limitation and thus flower sites and associated fruit production in the next season.

If this biennial pattern would be no more than an oscillation of yields around a constant long-term average the phenomenon would not be much of a problem (other than causing some variability in yields). However, as we showed high fruit loads in one year may compromise subsequent yields also by inhibiting leaf growth and by consuming leaf nitrogen. Both go at the expense of future tree growth and could thus lead to longer term declines in yields. As our data indicate, proper management of fruit loads can prevent this from happening and is a management practice worth promoting to coffee farmers in order to produce coffee beans with higher physical and organoleptic quality. Reduction in nitrogen content per unit leaf area and the associated reduction in leaf photosynthetic rate (A_{max}) due to the relative strength of fruits over vegetative sinks is a new insight reported in this thesis. The result clearly demonstrated that the decrease in A_{max} with high fruit load could not simply be associated with the straightforward feedback of sink demand or accumulation (e.g. Franck et al. 2006). In Box 1, a simplified relational diagram is presented, showing the fruit load effect on coffee tree leaf expansion and branch growth.

Bean quality

The study further showed that coffee bean quality is responsive to environmental and management practices and proved the postulate that measures and conditions that supply ample substrate per bean growth also improve coffee bean quality (Chapter 5). Both shading and fruit thinning increase source to sink ratio, enhance long-term substrate supply per bean and reduce competition among the developing beans. The effect of shading is probably indirect. Shading reduces canopy and fruit temperature as compared to exposure to full sun. Lower temperature provides a longer bean filling period. Higher altitudes also provide similar opportunities so that bean filling takes relatively longer time and improve bean quality.

Indeed, we showed that in a study comparing bean quality of different genotypes across altitudes, altitude explained 46 to 60% of the total variation in total coffee quality, with high altitudes being associated with lower temperatures. Coffee beans from higher altitude were



Box 1. Relational diagram showing fruit load effect on coffee tree leaf expansion and branch growth. (Forrester symbols according to Visogrind)

The relational diagram shows effect of fruit load on coffee trees leaf expansion, branch growth and assimilation rate. The assimilation rate is dependent on photosynthetically active radiation (PAR), leaf area index (LAI), the light extinction coefficient (k), leaf nitrogen content (Na (g m^{-2})) and the radiation use efficiency (RUE). LAI is determined by leaf dry weight and SLA. Carbon assimilates are partitioned to fruits (Pf), leaves (Pl) and branches (Pb). FDM, BDM and LDM represent the dry weights of fruits, leaves and branches, while the corresponding growth rates are labelled FDMPR, BDMP and LDMP, respectively; **Fnr** denotes the number of fruits per tree, i.e. fruit load. Reduced partitioning to leaves and branches when more fruits are developing reduces growth and expansion of these organs. Increased number of fruits removes more nitrogen from leaves and enhances senescence and death of leaves. Leaf nitrogen limitation has a negative feedback on assimilation rate. Reduced rate of branch extension in the presence of high fruit numbers limits sites and positions for new flower bud development, conditioning for low yield in the next season.

more acidic with higher body and flavor characteristics which are much more preferred by consumers than beans from mid or low altitudes (Chapter 5). Notably altitude had a larger effect on quality than genotype.

Overall, we showed that nitrogen application, shading, fruit number regulation and higher altitude could all significantly contribute to bean quality improvement. Fruit load management gave the highest gain (0.61 points on a scale of 6 to 10 for the average of organoleptic attributes), while an increase in 600 m in altitude resulted in average gain of 0.44 (Table 4).

Arabica coffee productivity is tightly linked to climate variability (Camargo 2010) and is thus influenced by natural climatic (e.g. temperature and rain fall) variability. Continues exposure to higher temperatures leads to tree stress, growth depression and bean quality loss. It has been forecasted that sustainability of the coffee industry faces serious challenges in the coming decades (Gay et al. 2006; Jaramillo et al. 2011). Due to the ongoing climate change, model calculations also showed that 65 – 100% suitable area for Arabica coffee production in the country will be lost in the year 2080 (Davis et al. 2012). Our results (discriminating 0.25 points on the scales from 6 to 10) indicate quantitatively the relation between increase in temperature and quality loss (0.30 points loss per ^{o}C higher temperature). Warming means fewer cool coffee growing areas in the country often leading to the loss of beverage quality. At altitudes, which are below the ideal level, warming will worsen productivity and quality. It will become more difficult to achieve the same crop stand, productivity and quality compared to areas that are climatically suitable for coffee. This change in climate probably pushes farmers to shift to suitable areas or urges them to change their coffee production strategies. The strategies will require the use of agroforestry systems as potential adaptive strategy for maintaining coffee productivity and genetic resources or a shift from Arabica to Robusta, the latter is more suited to harsh climatic conditions (Nair 2010).

Application for model development

Quantitative process-based models (e.g. SUCROS and GECROS) have been developed for different crops to understand the physiology of individual plant organs and their developmental processes (Yin and Van Laar 2005). The models link physiological processes and morphological structures to predict biomass production under varying environmental factors. This study quantitatively described physiological traits: radiation use efficiency and light extinction coefficient of coffee trees, quantified proportion of dry matter and nitrogen partitioned to different organs greatly contributing to the development of such quantitative models for coffee trees. The study also quantitatively described coffee yield based on tree biomass. This is also another important contribution to coffee production. The collected information bridges gaps and can set the development of quantitative models predicting growth and production of coffee trees in motion. Detailed insights on branch and leaf appearance and development rates, leaf longevities and geometrical variables are essential traits in 3D architectural and functional structural plant model developments. For effective prediction of coffee growth dynamics, future research work should therefore focus on the determination and quantitative description of these important variables.

Concluding remarks

This study unravelled coffee trees biomass, yield and quality responses in relation to different measures and agronomic management practices. We addressed to what extent the measures and factors analysed in the study influence coffee tree performance. We explored the traits that underlie the patterns of responses and examined the extent to which the factors determine coffee biomass production, bean yield and the associated bean quality. The variation in biomass production and yield among coffee trees is due to the variation in light interception, radiation use efficiency, nitrogen uptake and utilization. Low nitrogen conditions limited leaf size expansion, affected coffee growth via reduction of the intercepted radiation and low radiation use efficiency. Nitrogen supply, on the other hand, increased RUE with larger increase the higher the amount of incident radiation. Apparent nitrogen recovery, ANR, of coffee trees is generally low and needs further attention. Shading and fruit thinning increased source to sink ratio while nitrogen nutrition enhances coffee trees vigour. When more assimilates are available to individual beans, more dry matter is accumulated and beans develop to their potential sizes, which enhances quality. Shading and higher altitude result in lower temperature of the fruits, allowing longer bean maturation and slower bean ripening. These ultimately allow beans to accumulate more dry matter and improve bean quality. For altitudes, which are below the ideal level, however, shade may be required to sustain quality.

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Summary

Ethiopia is heavily dependent on coffee and the crop is an important source of foreign currency for the country. Despite the natural endowment of the country and the economic importance of the crop to Ethiopia, both the value and the volume of coffee exported from the country declined substantially in the past few decades. An improvement in coffee productivity and quality has a direct impact on the livelihoods of a large number of resource-poor people. This calls for concerted efforts to improve both volume and quality of coffee and is expected to result in increased flow of export revenue to the country.

Appropriate management practices along with a better understanding of the crop behaviour are important requirements for improving growth, productivity and bean qualities of coffee trees. In coffee production, crop management includes, but is not limited to, decisions on the degree of exposure of the trees to sunlight (shade management), plant nutrition, particularly N supply, and choice of variety. Coffee is vulnerable to biennial bearing, i.e. alternating seasons of high and low yield per tree. The underlying mechanisms need further study, providing a basis for management intervention. Growth and yield responses to factors and conditions are important for the coffee grower, but so is the effect on bean quality. Bean quality is important on a competitive world market for coffee. To contribute to the improvement of coffee productivity and bean quality, this thesis analysed how variation in radiation and nitrogen fertilizers and fruit load affected coffee tree growth, yields and bean quality. The study also analysed how quality attributes are affected under different environmental conditions. As such, it also contributed to an increased morphological and physiological understanding of the processes that determine coffee tree performance.

Both radiation and nitrogen supply play pivotal role in coffee tree growth and production. To further evaluate their effects, experiment (Chapters 2 and 3) with four levels of radiations (30 -100% full sun) factorially combined with four rates of N supply (0 - 88 g tree^{-1} y^{-1}) and three varieties was conducted at Jimma, Ethiopia. Vegetative growth and development of plants were monitored from the age of 14 months to 507 days. First bean yields were measured 883 days after transplanting. More nitrogen was utilized and more biomass was produced the more radiation was available. Both the number of leaves produced and their size increased with N supply. As a result plants that had higher N also captured more light.

Coffee trees that grow with larger nitrogen supply also exhibited higher nitrogen content per unit leaf area (N_a), and associated to that, a higher rate of light-saturated

photosynthesis (A_{max}). The ability of coffee trees to convert the intercepted radiation to dry matter (radiation use efficiency, RUE) thus also improved at larger nitrogen input with larger positive effect the higher the radiation level (positive interaction of nitrogen and radiation on RUE), resulting in larger tree biomass compared to trees in low radiation environment. Quantitative information on RUE in coffee is new.

Coffee trees exhibited morphological and physiological plasticity improving their performances in a range of radiation environments. Light extinction coefficient decreased with increase in radiation level, indicating that trees adjusted their leaf orientation to high light exposure allowing for a more uniform light distribution among their leaves. Trees also increased A_{max} in response to increased radiation. Trees in low radiation environment increased their main stem extension and specific leaf area. These responses are manifestations of the 'shade avoidance syndrome', which in general allows trees to overtop the neighbouring plants and intercept a larger proportion of light under the overstory.

Radiation and nitrogen supply also had positive interactive effects on nitrogen uptake and utilization. At a given nitrogen supply, both nitrogen uptake and utilization increased with radiation. This is likely because larger radiation interception per plant enlarges the carbon resources for growth and the requirement for nitrogen and makes more assimilates available for roots. Conversely, limiting nitrogen availability reduces the growth potential. Compared to other crops, however, the apparent nitrogen recovery of coffee trees is generally low (7 to 17%). Low fertilizer use efficiency implies large risk of N losses to the environment, and thus more research is needed to better understand the reasons for this low ANR and the fate of applied nitrogen fertilizer.

There were also positive interactions between radiation and nitrogen supply on coffee bean yield per tree. Radiation tended to increase yields but much more so at high than at low N application. Traits underlying larger biomass production (e.g. RUE) are equally important for bean yield improvement per tree. Based on a cost benefit analysis, It was estimated that an average of 76 g N per tree per year would be the optimal fertilizer rate in the sense that it provides the largest net benefit of coffee production. This result was independent of the radiation level indicating that radiation strongly affects nitrogen uptake and use efficiency.

An important characteristic of coffee trees is poorly self-regulation of their productivity, resulting in a so-called biennial pattern in fruit production. To evaluate effects of fruit load on vegetative growth, bean yield and coffee bean quality, fruit load manipulation (25, 50, 75 % and controls keeping 100 % of the fruits per tree) on coffee trees were examined in two field experiments in 8-years old, shaded plantations over two successive years (Chapter 4). Higher fruit load in a given year, reduced vegetative growth, reduced bean quality and resulted in a strong reduction in fruit bearing in the next year (stronger biennial bearing). The relative strength of fruits over vegetative sinks also reduced nitrogen content per unit leaf area, N_a, and the associated leaf photosynthetic rate (A_{max}). Thinning some fruits at their young stage of development modulated the competition among the competing sinks, stimulated branch extension, new flower positions and new leaf development. These reduced biennial bearing in coffee and contributed to higher yields, aggregated over two seasons. The practice is thus a management intervention worth promoting to coffee farmers in order to regulate coffee productivity, reduce biennial bearing and produce beans of higher quality.

Coffee bean quality is a sum of favourable characteristics that satisfies requirements of different actors in the coffee chain and is the factor determining its price in the coffee market. In this study coffee bean quality (Chapter 5) was measured in the experiments described in Chapters 2, 3, and 4. In addition, quality was measured in the first bean yield of an experiment with six varieties grown at three altitudes (1410, 1650 and 2063 m asl). Nitrogen application, shading, fruit load manipulation and higher altitude all significantly contributed to bean quality improvement. Nitrogen application enhanced coffee trees vigor while shading and fruit thinning increased source to sink ratio. The management interventions enhanced supply and accumulation of assimilates per individual bean, and thus beans developed to their potential sizes. Bean quality also increased with the altitude at which trees are grown, which was likely due to a negative temperature-quality relationship. Coffee bean quality increased with decreasing growing temperature. The results are in line with the hypothesis that coffee bean quality is promoted by factors and conditions that support non-limiting supply of resources for beans to grow and by a sufficiently long period of maturation. For altitudes, which are below the ideal level, shade may be required to compensate for quality.

In general, the study indicated that there are different agronomic, environmental and tree management options for increasing coffee tree productivity and improving coffee bean quality. For countries like Ethiopia, where ordinary labour is cheap, the low-tech management practices (such as fruit thinning) may be economically the most feasible. Fruit thinning particularly improves coffee quality and prices for high coffee quality can be more than twice the market price of ordinary coffee quality. Insights in the fate of nitrogen not taken up and good estimation of leaching losses are generally needed to evaluate productivity against environmental quality. For organic coffee producers estimates of mineralization allow assessing the degree of nitrogen limitation in their production system.

Quantitative description of morphological and physiological traits underlying tree performance is a significant contribution of this study. Further exploration of the potentiality of these traits is a virtue offered by quantitative crop growth models that capture each response and predict the behaviour of coffee tree and its fitness in different agro-ecosystems. Further work is needed on appearance and development rates of organs (e.g. branches, leaves), their longevities and the geometric variables in order to develop a dynamic 3D functional-structural coffee tree model.

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List of publications

Bote AD, Vos J (2016) Branch growth dynamics, photosynthesis, yield and bean size distribution in response to fruit load manipulation in coffee trees. *Trees*:1-11

Akassa B, Belew D, **Adugna D** (2014) Effect of Inter and Intra Row Spacing on Potato (Solanum tuberosum L.) Seed and Ware Tuber Seedling Emergence and Establishment at Bako, Western Ethiopia. *J Agron* 13:127-130

Akassa B, Belew D, **Adugna D** (2014) Yield and Quality of Potato (Solanum tuberosum L.) Seed Tuber as Influenced by Inter and Intra Row Spacing at Bako, Western Ethiopia. *J Biolo Sci 14:431-435*

Bote AD, Vos J Fruit Load, Yield, Vegetative Growth and Photosynthesis in Coffee (*Coffea arabica* L.). In: 25th International Conference on Coffee Science, Armenia, Colombia, 8 - 13 September 2014, pp. 42-45

Fayera A, **Adugna D**, Mohammed M (2014) Evaluation of Teff [Eragrostistef (Zuccagni) Trotter] Responses to Different Rates of NPK Along With Zn and B in Didessa District, Southwestern Ethiopia. *World Applied Sciences Journal* 32:2245-2249

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Yonas M, Garedew W, **Adugna D** (2014) Multivariate Analysis among Okra (Abelmoschus esculentus (L.) Moench) Collection in South Western Ethiopia. *J Plant Sci* 9:43 - 50

Yonas M, Garedew W, **Adugna D** (2014) Variability and Association of Quantitative Characters among Okra (Abelmoschus esculentus (L.) Moench) Collection in South Western Ethiopia. *J Biol Sci* 14:336 - 342

Kassaye T, **Adugna D,** Getahun L (2013) Evaluating seedling establishment of tomato (lycoperscum esculentummill.) varieties as influenced by nacl stress. *Intern J Current Agric Sci* 3:10-14

Teshome K, **Adugna D**, Garedew W (2013) Effect of Drying Temperature and Duration on Biochemical Composition and Quality of Black Tea (Camellia sinensis L.) O. Kuntze at Wush Wush, South Western Ethiopia. *Asian J Plant Sci* 12:235

Yohannes KW, Belew D, **Adugna D** (2013) Effect of Farmyard Manure and Nitrogen Fertilizer Rates on Growth, Yield and Yield Components of Onion (Allium cepa L.) at Jimma, Southwest Ethiopia. *Asian J Plant Sci* 12:228 - 234

Bote AD, Struik PC (2011) Effects of shade on growth, production and quality of coffee (*Coffea arabica L.*) in Ethiopia. *J Horti Forestry 3:*336 - 341

Adugna D, Daba G, Bane D, Tolessa K (2011) Identification of major causes of postharvest losses among selected fruits in Jimma zone for proffering veritable solutions. *Int J Curr Res* 3:40-43

Girma K, Kassaye T, **Adugna D** (2011) Evaluating tomato responses: a means of screening varieties to saline soil. *Intern J Current Agric* Res 3: 1-11

Kebebew Z, Garedew W, **Adugna D** (2011) Understanding Homegarden in Household Food Security Strategy: Case Study Around Jimma, Southwestern Ethiopia. *Res J App Sci 6:38-43*

Mitchell C, Belew D, **Adugna D**, Muleta D, Fikreyesus S (2010) “The farmer and her husband”: Engendering the curriculum in a Faculty of Agriculture in an Ethiopian university. *Agenda* 24:66-77

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Eco-physiology of coffee plant, shade and fruit thinning management in coffee, effect of N availability on coffee dry matter production, coffee bean chemical compositions as effected by shade and nitrogen rates, coffee source-sink manipulation, sustainable coffee production and coffee quality

Writing of project proposal (4.5 ECTS)

- Examining Ethiopian coffee quality in relation to environment, resources, tree management and procedures in the coffee chain

Post-graduate courses (4.2 ECTS)

- Introduction to R for statistical analysis; PE&RC / SENSE (2011)
- Bayesian statistics; PE&RC / SENSE (2011)
- The art of modelling; PE&RC (2015)

Laboratory training and working visits (1.2 ECTS)

- Coffee processing and quality analysis; Douwe Egberts (2011)
- Nitrogen analysis, GC and HPLC training; JEJE Laboratory ,Addis, Ethiopia (2012)
- Coffee quality analysis; ECX-Jimma, Ethiopia (2013)

Invited review of (unpublished) journal manuscript (2 ECTS)

- International Journal of Agricultural Science, IJAS: Study on the dry matter component of two cucumber varieties using farm yard manure (2012)
- African Journal of Agricultural Research, AJAR: Interactive effect of genotype, environment and processing methods in the chemical composition, expression and sensorial quality of arabica coffee (2016)

Deficiency, refresh, brush-up courses (12 ECTS)

- Plant plasticity and adaptation; PPH (2011)
- Ecological models and data in R; CSA (2011)

Competence strengthening / skills courses (2.4 ECTS)

- Scientific writing; WGS (2015)
- Information literacy; WUR Library (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Weekend (2011, 2015)

Discussion groups / local seminars / other scientific meetings (5.1 ECTS)

- PhD Seminar: virtues and vices of plant modelling: the need for modelling in plant systems biology (2011)
- Plant production system lunch seminar series (2011, 2015, 2016)

- Centre for crop system analysis seminar series (2011, 2015, 2016)
- The plant-soil interactions discussion group (2011, 2016)
- Plant science seminar: how realistic is our "two times more with two times less" ambition? (2012)
- Monthly CSA PhD meetings (2015, 2016)
- Sustainable intensification of agricultural systems group (2016)

International symposia, workshops and conferences (6 ECTS)

- Learning to improve coffee quality; Wotro workshop, Jimma, Ethiopia (2009)
- The 25th conference on coffee science; Armenia, Colombia (2014)
- 5th Annual research conference of Jimma University, Jimma, Ethiopia (2014)
- X International symposium on modelling in fruit research and orchard management; Montpellier, France (2015)

Lecturing / supervision of practicals / tutorials (6 ECTS)

- Horticultural crop physiology; Jimma University (2013)
- Plant physiology; Jimma University (2014)

Supervision of MSc students (12 ECTS)

- Effect of shade levels and rates of nitrogen fertilizer on morpho-physiological performance of different coffee cultivars (Coffea arabica l.) at Jimma, South Western Ethiopia
- Analysis of Genotype by Environment Interaction on Growth and Gas Exchange Responses of Arabica Coffee (Coffea arabica L.) at different altitudes, South Western Ethiopia
- Evaluation of some CBD resistant coffee (coffea arabica L.): genotypes for morphological and physiological characteristics at different altitudes of manna woreda at South Western Ethiopia
- Effects of nitrogen nutrition and shade on leaf photosynthesis of two coffee varieties (Coffea arabica L.)

Curriculum vitea

Adugna Debela Bote was born on 22 November 1976 in East Wollega Shambo, Ethiopia. He took his BSc degree in Horticulture from Jimma University College of Agriculture in 2003 and was recruited as graduate assistant I in the same college where he took his BSc. In 2005, he joined Wageningen University for his postgraduate study with a scholarship from Nuffic and graduated in 2007 with MSc degree in Organic Agriculture (MOA). From 2008 to 2011, the author served as head of the department of Horticulture and Plant Sciences, Jimma University and contributed in the development of different programmes in the department. In September 2011 again with a scholarship from Nuffic, he joined back Wageningen University for a PhD study entitled “Examining growth, yield and bean quality of Ethiopian coffee trees: towards optimizing resources and tree managements”. In this study, he analysed how coffee trees responded to different resources, tree management and also the growing environments.

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