The relationship between elevation, soil temperatures, soil chemical characteristics and green coffee bean quality and biochemistry in southwest Ethiopia

Merkebu Getachew[ab] (merkeb2020@gmail.com), Kassaye Tolassa[c], Pieter De Frenne[a], Kris Verheyen[a], Ayco J.M. Tack[d], Kristoffer Hylander[d], Biruk Ayalew[d], Pascal Boeckx[e]

[a]Forest and Nature Lab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Belgium
[b]Department of Horticulture and Plant Sciences, College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia
[c]Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia
[d]Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden
[e]Isotope Bioscience Laboratory, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

Abstract

Green coffee bean quality and biochemistry are influenced by environmental variables. The present study was designed to study the influence of soil temperatures and soil chemistry on bean physical attributes, bean quality (assessed by three internationally trained, experienced and certified Q-grade cuppers licensed by the Specialty Coffee Association (SCA) Coffee Quality Institute (CQI) and biochemistry of green coffee beans). The study was performed in 53 farms in southwest Ethiopia distributed along an elevational gradients (1500 – 2160 m a.s.l.) and with varying shade canopy cover (open to dense shade). A total of 159 individual coffee trees were sampled. Shade tree canopy cover, soil temperature, and soil chemistry as well as coffee management intensity were quantified as explanatory variables. Green bean quality was negatively correlated to soil temperatures. On the other hand, hundred bean mass and green bean biochemistry (caffeine, trigonelline and chlorogenic acid contents) were negatively correlated to soil temperatures but positively to soil chemistry. During the coffee fruit development period (flowering to fruit maturity), temperature appeared to be a driving factor influencing coffee bean quality and biochemistry. Total specialty quality was significantly associated with soil chemistry , in which 84% of the variation could be explained by soil chemical variables. This study is the first to demonstrate the relationship between soil temperatures and chemistry in coffee bean quality and green bean biochemical compositions. Although the relative importance of factors such as air temperatures and humidity and soil moisture are missing from this study, we find that soil temperatures and soil chemistry have a strong effect on coffee bean quality and biochemistry. Overall, climate change, which generally involves a substantial increase in mean temperatures of tropical regions, could be expected to have a negative impact on coffee bean quality and biochemistry.

Keywords: Arabica coffee, Ethiopia, cup quality, soil properties, microclimate

1. Introduction

Coffee is one of the most important global commodities providing livelihood opportunities to millions of people in the global South (Legesse 2020; Ovalle-Rivera et al. 2015; Davis et al. 2012). In addition to being an important cash crop to farmers in Ethiopia, coffee generates about a quarter of the country's export earnings (Davis et al. 2012). Southwestern Ethiopia is known as a primary center of origin and diversity of Arabica coffee (hereafter referred to as coffee), where the species grows naturally as understory shrub in moist Afromontane forests (Hundera et al. 2013; Davis et al. 2012). Coffee in this region is mainly grown under natural shade trees or in farming systems that deliberately incorporate specific shade trees. Common coffee shade tree species in this region include *Albizia gummifera, Acacia abyssinica, Cordia africana, Croton macrostachyus,* and *Millettia ferruginea.*

Coffee bean quality is mainly described by its physical attributes, cup quality (sensory quality attributes) and chemical bean constituents (Cheng et al. 2016; Dos Santos Scholz et al. 2016). Cup quality, determines the desirability of a coffee for consumption which impacts the price setting and competitiveness in the international markets (Tolessa et al. 2018). These quality attributes, such as cup cleanness, acidity, body and flavor can be distinguished by sensory organs and are assessed by professional cup tasters based on established procedures. Green bean chemical constituents like caffeine, trigonelline and chlorogenic acids (CGA) are the dominant precursors for coffee aroma and flavor development and these chemical compounds determine the flavor, aroma and bitterness, and ultimately the quality of the final coffee beverage (Chemura et al. 2021; Cheng et al. 2016; Bertrand et al. 2012; Joet et al. 2010). On the other hand, bean physical attributes are mainly determined by mass, defects, length and diameter (Teklu et al. 2011).

Regional warming and increasingly erratic rainfall have already increased the frequency of poor harvests, affecting coffee productivity (DaMatta et al. 2018; Ramalho et al. 2018; Laderach et al. 2017; Craparo et al. 2015). Rising temperatures, droughts, and erratic weather patterns are predicted to reduce the overall land suitable for growing Arabica coffee in Ethiopia by 50% between 2040-2070 primarily in the Harar, Sidamo and Jimma areas (Moat et al. 2017). Nearly half of the current coffee growing areas of the country would lose 20-40% climate suitability, mainly in areas of low to medium elevations (Ovalle-Rivera et al. 2015; Davis et al. 2012). The productivity and quality of coffee depends on complex spatiotemporal interactions of climatic, topographic, edaphic and biological components (DaMatta et al. 2019; Cerda et al. 2017), which complicates the prediction of climate change impacts. Hence, in the context of the observed and predicted climate changes, continuous adaptation will remain a significant challenge for coffee production and bean quality.

The spatial distribution of air temperature is controlled by elevation (Navarro-Serrano et al. 2020). Likewise, soil temperature is the function of heat flux in the soil as well as heat exchanges between the

soil and atmosphere (Elias et al. 2004). Although soil temperatures are affected by the interaction of multiple factors like soil inputs, mulching, irrigation, air temperature was found to be the main driving variable for the changes in soil temperatures (Onwuka and Mang 2018; Barman et al. 2017). Coffee trees growing at high elevations produce beans with greater bean quality because elevation affects coffee quality via the growing temperatures (Avelino et al. 2007; Muschler 2001). Lower temperature slows down bean ripening processes and allows more time for bean filling (Muschler 2001). Literature relating soil temperatures with coffee bean quality and biochemistry are rarely available and hence, our main focus was given to measure this variable. Meanwhile, soil temperature is an important environmental variable but it is a rarely reported indicator of climate change. In this regard, the ideal growing temperature of Arabica coffee was appeared to be between 18 and 21°C (Camargo 2010) and temperatures outside this range will cause incomplete maturation. Effect of elevation on biochemical composition of green coffee beans has also been demonstrated in that a higher caffeine content with the increasing elevation, while chlorogenic acids content decline (Avelino et al. 2007). Bertrand et al. (2012), on the other hand, reported a higher chlorogenic acid content at higher elevations than at the low. Meanwhile, soil nutrients perform specific and essential functions in plant metabolism, and their deficiency may lead to metabolic disturbances that can affect coffee bean quality and biochemistry. These soil nutrients are influenced by environmental changes particularly, temperature changes might have a pronounced effect on soil nutrient dynamics which might have effect on coffee bean quality and biochemistry (Clemente et al. 2015).

There are several alternatives to reduce the negative impacts of high temperatures on coffee production and to produce high-quality beans. One of the option is moving its cultivation areas to higher elevations as suitability moves upslope to compensate for the increased temperatures (Moat et al. 2017; Ovalle-Rivera et al. 2015). The other option is via managing the canopy shade and the associated cooling of the understorey microclimate (De Frenne et al. 2019, Davis et al. 2012). Shifting coffee-growing areas upslope might bring conflicts with land uses with crops other than coffee (Magrach and Ghazoul 2015). Besides, it can induce adverse socio-economic and environmental impacts associated with deforestation for new coffee cultivation (Meyfroidt et al. 2013). Open land at high elevations might be remote or too steep for growing coffee (Bunn et al. 2015; Rahn et al. 2014). The latter option (manipulating sub-canopy microclimate), could be more realistic opportunity to modulate the increasing air temperatures (Ruslim et al. 2017; Ovalle-Rivera et al. 2015; Davis et al. 2012), considering elevation as a space-for-time substitute for climate warming (De Frenne et al. 2013). Meanwhile, there are also some trade-offs and adverse effects of shade where the decrease in crop yield and quality is likely to occur as the trees canopy cover increases due to the competition for light, water, and nutrients (Sarmiento-Soler et al. 2020; Blaser et al. 2017).

Although the effect of elevation and shade canopy cover on coffee bean yield has been well studied, information on the effects of soil temperatures and soil chemistry on cup quality and green bean biochemistry is scarce. A few available studies suggest that elevation and shade influence green coffee bean quality and biochemistry when they were treated independently or in combination (Worku et al. 2018; Leonel and Philippe 2007; Avelino et al. 2007). Certain studies reported that more shaded conditions reduce temperature stress in the canopy and prolongs the maturation period of coffee berries. It also reduces periodic over-bearing and a subsequent die back of coffee plants (Leonel and Philippe 2007). Likewise, higher shade promotes slower and more balanced fruit maturation by the mother plant, thus yielding a better-quality product than unshaded coffee plants at lower elevations (Barbosa et al. 2012; Geromel et al. 2008; Leonel and Philippe 2007; Muschler 2001). On the other hand, larger bean size and mass were obtained when canopy cover increased from 0 to 80%. A recent study from southwest Ethiopia indicated that dense shade at high elevations produced beans with lower cup quality, whereas higher shading at lower elevations increased cup quality (Tolessa et al. 2017). Another recent study from Ethiopia revealed that coffee plants grown under shade produced greater acidity than those grown under the full sun (Worku et al. 2018). Besides, recent studies from southwest Ethiopia revealed that soil fertility parameters affected cup quality of wild Arabica coffee in its natural habitat and that available P, K and the ratio between Mg and K were the most important soil chemical variables that influenced bean size, cupping scores, and green bean biochemistry (Yadessa et al. 2020; Clemente et al. 2015).

Here we examined how soil temperatures and soil chemistry affect green coffee bean biochemical composition, hundred bean mass and sensory quality attributes. The following research question was specifically addressed: how does shade tree canopy cover influence coffee bean physical attributes, brew quality, and green bean biochemistry along elevational gradients via its impacts on the soil temperature and chemistry? We hypothesized that:

1. Cooler temperatures lead to better bean quality and higher caffeine, trigonelline, and chlorogenic acids content.

2. Soil fertility has a positive effect on bean quality and has a higher potential to improve caffeine, trigonelline, and chlorogenic acids content.

3. Materials and Methods

Study area

The study was conducted in the Goma and Gera districts of Jimma Zone in southwestern Ethiopia (7°37'48'' – 7°56'37''N latitude and 36°13'41'' – 36°39'17''E longitudes). In total 53 coffee farms along an elevational gradient ranging from ca. 1500 to 2160 m asl were selected. The region is characterized by a humid and warm subtropical climate with a yearly rainfall between 1500 and 2000 mm. The main rainy season is from May to September (monomodal rainfall), accounting for about 85% of the annual rainfall. Coffee cultivation in the region is rain-fed. Soils are classified as Eutric Nitisols, which are

deep and well-drained soils with a clay content of more than 30% and a pH (measured in H_2O) between 4.2 and 6.2 (Kebede et al. 2018; Kufa 2011).

Coffee plot selection and characterization

The study included agroforestry sites distributed across a landscape, comprising approximately 2500 km^2 area. In order to encompass an elevational and shade tree canopy cover gradient, 53 coffee farms (Fig 1) along an elevational gradient ranging between 1500– 2160 m asl. were selected. The sites vary considerably in their coffee management intensity ranging from little human interference to intensive management, including herbicides and mineral fertilizers, for the purpose of increasing coffee yield (Zewdie et al. 2020). The farms are also characterized by dense and diverse shade tree canopy cover. In the semi-plantation coffee production system, there is high anthropogenic disturbance resulting in a relatively species poor canopy consisting of tree species such as *Albizia schimperiana*, *Albizia gummifera* and *Croton macrostachyus*. Mulching and organic fertilizers are commonly used soil fertility management strategies. On the other hand, a plantation coffee system represents shade plantations with only few and large canopies. Shade trees are mainly indigenous species (most dominantly *Acacia spp*. and *Croton macrostachyus*), though recently, fast-growing exotic species have been introduced such as *Grevillea robusta*. High coffee stem density is also assumed to be a component of this production system. Herbicides and commercial fertilizers are applied regularly and mulching is also a common practice (personal observation). Accordingly, management intensities were classified into a categorical variable as plantation (n=8) and smallholder farms (n=45). To avoid spatial autocorrelation, the selected farms were at least 3-4 km apart. Sampling was conducted in 30 x 30 m coffee plots per farm, in which three coffee trees were selected from each coffee farm giving a total of 159 coffee trees. The selected three coffee trees were spread within the 30 x 30 m coffee plots at each site, and all of them were consistently positioned inside the plantation to avoid edge effects. The study was conducted from February to December 2019.

Environmental drivers

The following environmental variables were measured to describe elevation, shade tree canopy cover, soil temperature, and soil properties per coffee tree. The elevation of each coffee plot was measured with a Global Positioning System (GPS) (Garmin-60). Hemispherical photography was used to quantify shade tree canopy cover. Pictures were taken looking upwards from the center above each coffee tree, using a hemispherical fish-eye lens with the camera (Canon DS126091, ICES-003, Sigma 4.5 mm lens, Canon, Japan). Three photos were taken oriented towards the North. All pictures were taken under solidly overcast sky between 09:00 and 16:00. The analysis was performed in the blue color channel to improve the contrast between tree branches, foliage and sky (Schleppi et al. 2007; Juncker et al. 2004). The pictures were processed and analyzed using Gap Light Analyzer (GLA) (Jarcuska 2008; Jonckheere. et al. 2004; Frazer et al. 1999).

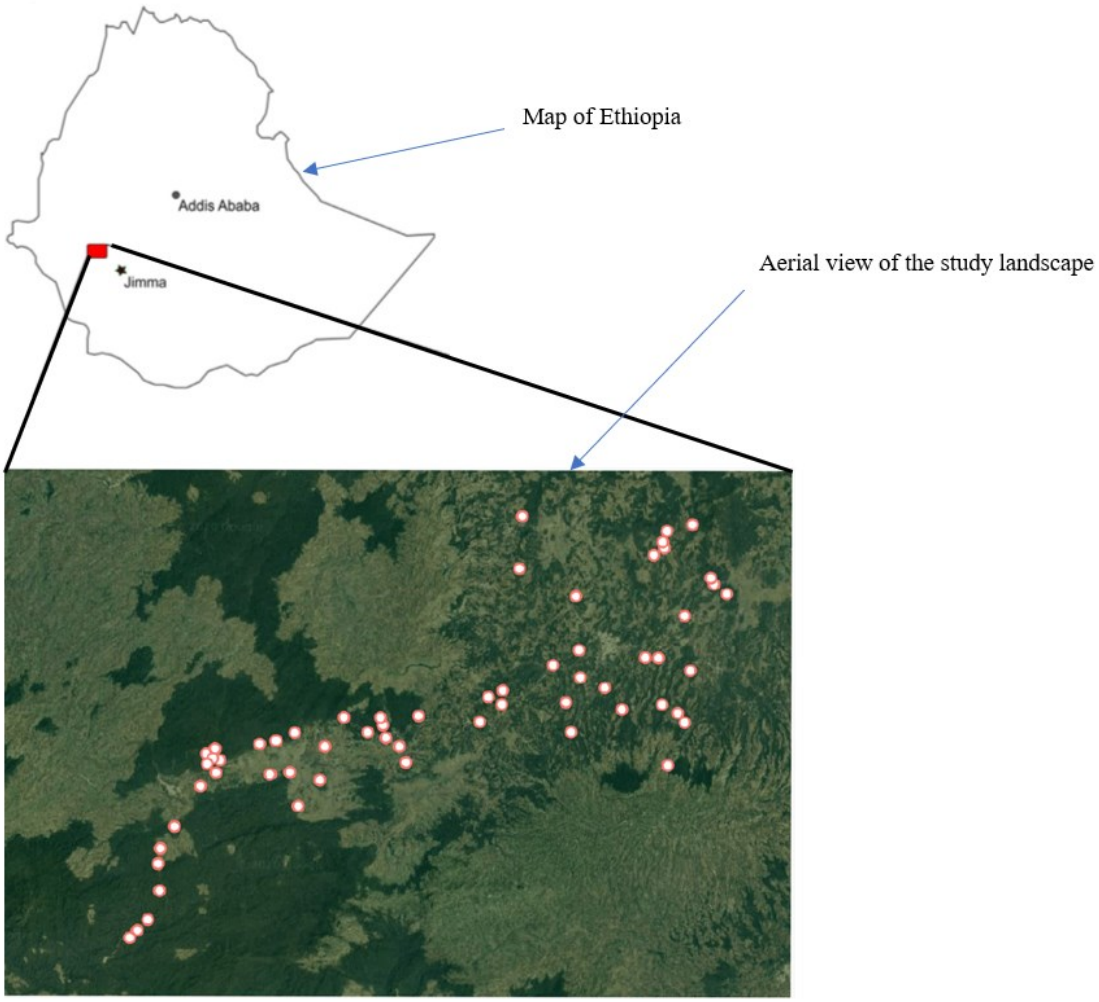

Fig. 1. Study area showing an aerial view of the study landscape representing the distribution of coffee farms established within the landscape; the dots represent coffee farms (both smallholders and commercial plantation).

As a proxy for microclimate, soil temperatures were recorded in each coffee farm at a three hour intervals between February and December 2019 (period in which coffee been development from flowering onwards takes place) using miniature temperature sensors iButton (DS192H, Maxim/Dallas Semi-conductor Corp., USA) buried in the soil at 10 cm depth and at 40 cm distance from the coffee tree trunks. We could not measure air temperatures because of theft of visible devices. The daily minimum, mean and maximum temperature were computed to ensure the best representation of temperature experienced by the coffee plant. The daily mean temperatures were calculated as the average of the daily maximum and minimum temperatures.

For each soil sample, an oven-dried sub-sample was used for the measurements of soil carbon, total N, Olsen-P, Ca, Mg and K. All the soil samples were dried to a constant weight at 65°C for 48 h, ground and sieved over a 2 mm mesh. For soil total C and N, the soil samples were combusted at 1200°C and the gases were measured by a thermal conductivity detector in a CNS elemental analyzer (Variomax Cube, Elementar, Germany). Olsen-P, which is a measure for plant-available P (Gilbert et al. 2009) was extracted in $NaHCO_3$ (ISO 11263:1994) followed by calorimetric measurement according to the

malachite green procedure (Lajtha et al. 1999; Robertson et al. 1999). Soil total Ca, K and Mg was measured by atomic absorption spectroscopy after the destruction of the soil samples with $HClO_4$ (65%), HNO_3 (70%) and H_2SO_4 (98%) inteflon bombs for four hour at 150°C. Exchangeable K^+, Ca^{2+}, Mg^{2+}, Na^+ and Al^{3+} concentrations were measured by atomic absorption spectroscopy (AA240FS, Fast Sequential AAS) after extraction in 0.1 M $BaCl_2$ (NEN 5738:1996).

Coffee berry sampling and measurements

All fully ripe, red-colored coffee berries were hand-picked once at peak harvest between October and November 2019 from each selected coffee tree using local coffee bags. Berries that ripe earlier were harvested first from lower elevation sites and subsequently the high elevation sites followed. The berries were dry-processed, i.e. sun-dried (on raised mesh wire) immediately after harvest (harvesting was in the morning and subjected to drying started in the afternoon). The berries were returned to traditional coffee bags before sunset and stored in clean rooms (to prevent spoilage) and were exposed to the sun in the morning until green beans attained 11.5% moisture content measured using coffee moisture tester (mini GAC, Dickey - John, USA). The berries were regularly turned to maintain uniform drying and the dried coffee berries were separately labeled and packed for analysis. The dried coffee berries were dehusked using a hulling coffee machine (coffee huller, McKinnon, Scotland) at Jimma University, cleaned and stored at room temperature.

Bean physical attributes: Bean length (mm) and diameter (mm) were measured using a bean measuring caliper (Mitutoyo, IP 67, CD-20-PPX, Kawasaki, Japan) using 10 beans per sample. Additionally, the mass of the beans was recorded by taking 100 beans from each sample. Finally, the green bean samples were submitted to the Ethiopian Commodity Exchange (ECX) for raw and sensory quality analyses.

Raw quality (40% of the total preliminary quality): A green coffee bean sample of 100 g was used for physical quality evaluation before roasting. Primary and secondary defects and odor, were assessed according to the procedures developed by the ECX (2011). The rating was based on a scale from 0 to 15 for the defects and 0 to 10 for odor.

Cup quality (60% of the total preliminary quality): Coffee bean samples were evaluated for cup quality attributes by a panel of three internationally trained, experienced and certified Q-grade cuppers in Jimma ECX center. Acidity, body, cup cleanness and flavor were assessed following a standard method (ECX 2011). This Q-grade standard method involves Q-certified cuppers, i.e., cuppers licensed by the Specialty Coffee Association (SCA) Coffee Quality Institute (CQI).

Roasting, grinding, and brew preparation: This was performed by the ECX laboratory in Jimma, Ethiopia. A roaster equipped with a cooling system, in which air was forced through a perforated plate, capable of roasting up to 500 g of coffee beans, was used for roasting the coffee beans. An amount of 100 g green beans was used for each sample and the beans were put into the roasting machine with six cylinders (Probat, 4 Barrel Roaster, Germany). They were carefully roasted for 7-8 minutes to medium roast at temperatures of 200°C. Subsequently, the roasted bean samples were ground to a medium level using a Guatemala SB electrical grinder, which were cleaned well after each sample. The medium roasted coffee was tipped out into a cooling tray and allowed to cool down for 4 minutes rapidly by blowing cold air through it. Then, eight grams of coffee powder was put into a 250 mL cup and 5 cups per coffee sample were used. Next 125 ml boiled water (93°C) was poured onto the ground coffee, followed by stirring the content to ensure homogeneity of the mixture. Then, the cups were filled with an additional 125 mL and left to settle. After three minutes, floating coffee was skimmed, and the brew was ready for cup tasting. Finally, the five prepared cups were cup tasted by three professional Q-grade cuppers operating in ECX. Each panelist gave their independent judgment using a cupping form and the average score of the three cuppers was used.

Finally, the total preliminary quality was calculated using raw and cup quality scores. Coffee samples of grades 1-3 (specialty 1, 2 and 3) were assessed for total specialty quality. Accordingly, aroma, flavor, acidity, body, uniformity, cup cleanness, overall preference, aftertaste, balance and sweetness were rated on a scale from 0 to 10. The sum of all these cup quality attributes gave a total specialty quality ranging from 0 to 100 (https://sca.coffee/research/protocols-best-practices).

Bean biochemistry: Caffeine, trigonelline and chlorogenic acid content were analyzed at the Ethiopian Institute of Agricultural Research as described by Vignoli et al. (2011). Ground green coffee beans (0.5 g) were submitted to direct hot water (95°C, 50 mL) extraction and stirred for 20 minutes on a hot plate. The extract was filtered through No. 4 Whatman filter followed by a 0.45 μm PTFE filter prior to injection into High Performance Liquid Chromatography (HPLC) (Surveyor, Thermo Finnigan, USA) having a prevail C18 (250 × 4.6 mm, i.d. 5 μm, 25°C) column. The mobile phase is composed of 5% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). Flow rate was 0.5 mL min^{-1} and the injection volume was 10 μL. Both caffeine and trigonelline acid were detected by UV detector at 272 nm wavelength, whereas chlorogenic acids were detected at 320 nm. The reported chlorogenic acid was the total chlorogenic acids content. In all the cases, the standards were used for quantification and retention time determinations.

Data analyses

As response variables, we retained six key variables representing the physical bean attribute (hundred bean mass), brew quality (total preliminary and specialty quality), and bean biochemical compositions (caffeine, trigonelline and chlorogenic acids content). First, the effects of elevation and shade tree canopy cover was assessed. Second, relationships with soil chemical variables and soil temperature were tested. Third, the link between green bean biochemistry and quality was examined.

Linear mixed-effect models (LMMs) were fitted for all analyses and response variables. First, elevation and shade tree canopy cover were used as fixed effect predictors and coffee farm as a random effect term. These models allowed us to explicitly model our structured and nested data, containing clusters of non-independent observational units that are hierarchical in nature (farms in this case). As coffee trees were clustered in different farms, the variation across these sampling farms is assumed to be random and uncorrelated with the predictor variables. Therefore, coffee farm was included as a random factor. Both full and reduced linear mixed models (LMM) were performed in which the full model consisted of elevation, canopy cover, and elevation-by-canopy cover interactions as fixed effects in linear mixed-effect models. We also included management intensity as a categorical fixed effect. All the data were presented to the tree level (n = 159). The models were then fitted using maximum-likelihood methods in the 'lme4' packages using the 'lmer' function (Harrison et al. 2018). The p-values of the fixed effects (elevation, canopy cover, and their interaction) and the overall model significance were estimated based on the denominator degrees of freedom calculated with the Satterthwaite approximation, in the 'lmerTest' package (Bates et al. 2018). Moreover, model assumptions were checked after fitting the models. For all models, the distributions of error terms (residuals) were approximately normal having a constant variance, with zero means, indicating adequate model fit (Nakagawa and Schielzeth 2013). To test the explanatory power of several different predictor variables for the variation in response variables, the coefficient of determination (R^2) was quantified using the 'r.squaredGLMM' function in the package 'MuMIn' (Barton and Barton 2015). Accordingly, both marginal and conditional R^2 values were determined to describe the proportion of variance explained by the fixed effects alone as well as the fixed and random factors together, respectively (Nakagawa and Schielzeth 2013).

Finally, we tested the effects of the environmental predictors (soil chemical characteristics and soil temperature). First Principal Component Analyses (PCA) were conducted to reveal groupings and relationships between the main environmental predictor variables for soil temperatures and soil chemical variables separately. To select prominent variables for subsequent regression analyses, the first two principal components from soil temperature variables were taken (Supporting Information Fig. S1). Likewise, the first two principal components for soil chemical variables were taken (Supporting Information Fig. S2). Then, the score values obtained from the above considered principal components were utilized as independent variables in LMM to identify the main driving variables determining bean

quality attributes and biochemistry using backward variable selection procedures. For the analysis of principal components, the function 'prcomp' was used from the packages 'factoextra' and 'stats'.

The R function 'fviz' was used to create a ggplot2-based visualization in the biplot analysis. The R fu nction 'ggplot' was used from the packages 'ggplot2' and 'broom' to produce plotting. To combine m ultiple graphics, the package gridExtra was utilized. The R version 3.6.1 (R Development Core Team 2019) was used for all analyses.

3. Results and Discussion

The total preliminary quality was used to classify the coffee samples into different quality grades. According to ECX (2011), dry-processed coffees can be categorized as follows based on total preliminary quality: 91-100 (grade 1), 81-90 (grade 2) and 71-80 (grade 3) whereas the specialty coffee achieving scores between 85-100 are classified as specialty 1 (Q_1) and 80-84 is specialty 2 (Q_2). In our present study, the minimum total preliminary quality was 71, and the maximum was 91, whereas the minimum specialty quality was 80.5 and the maximum score was 90.5. Hence, all our samples fell under grade 2 and 3, and Q_1 and Q_2 (Table 1).

Table 1. Fixed effects, coffee bean physical attributes, green bean biochemistry and total preliminary and specialty quality.

Environmental variable	Soil chemistry	Response variables	Descriptive statistics for 159 coffee trees		
		1. Bean physical attributes	Min	Mean	Max
Elevation (m)	pH (H_2O)	- Hundred bean mass (g)	9.4	15.2	19.9
Canopy cover (%)	Soil C (%)	- Bean length (mm)	8.0	9.1	10.4
	Soil N (%)	- Bean diameter (mm)	5.8	6.3	6.7
Soil temperature (mean,	Soil K (mg kg^{-1})	**2.** Bean biochemistry			
min and max) in ^0C	Soil Mg (mg kg^{-1})	- Caffeine (g kg^{-1} dry mass)	0.5	1.2	2.3
	Soil Ca (mg kg^{-1})	- Trigonelline (g kg^{-1} dry mass)	0.9	1.5	2.7
	Olsen-P (mg kg^{-1})	- Chlorogenic acids (g kg^{-1} dry mass)	3.8	7.4	14.1
		3. Cup quality attributes			
		3.1. Preliminary attributes			
		- Primary defects (score 0-15)	12.0	15.0	15.0
		- Secondary defects (score 0-15)	1.0	6.0	15.0
		- Odour (score 0-10)	10.0	10.0	10.0
		Raw total	23.0	30.9	40.0
		- Acidity (score 0-15)	9.0	12.2	15.0
		- Body (score 0-15)	9.0	12.0	13.0
		- Flavour (score 0-15)	9.0	10.2	15.0
		- Cup cleanness (score 0-15)	15.0	15.0	15.0
		Cup total	41.0	49.3	58.0

Total preliminary quality	71.0	80.3	91.0
3.2. Specialty attributes	(score 0-10)		
- Aroma	7.3	7.8	8.5
- Flavour	7.0	7.8	8.8
- Aftertaste	7.0	7.8	8.8
- Acidity	7.0	7.9	8.8
- Body	7.3	7.8	8.5
- Cup cleanness	10.0	10.0	10.0
- Overall	7.0	7.8	8.8
- Sweetness	10.0	10.0	10.0
- Balance	7.0	7.8	8.8
- Uniformity	10.0	10.0	10.0
Total specialty quality	80.5	84.6	90.5

Relationship between elevation, shade canopy cover and coffee bean quality and biochemistry

In order to link coffee bean quality and biochemistry to elevation and shade canopy cover, the associated soil temperature and chemical variables were considered separately as variables explaining changes in hundred bean mass, total preliminary and specialty quality. A clear relationship of hundred bean mass with elevation and shade canopy cover was observed, confirming that a greater hundred bean mass was produced in response to the increasing elevation at different shade levels. Hundred bean mass was increased under light shade (10-35% canopy cover) and intermediate shade levels (35-65% canopy cover) with elevation. The interaction explain 28% of the variation (Table 2). However, under dense shade (65-100% level), the hundred bean mass did not change with elevation (Table 2 and Fig 2a). Similarly, the interaction effect of elevation and canopy cover significantly influenced the total preliminary quality. An increased total preliminary quality was obtained at higher elevations under dense shade conditions while the total preliminary quality did not change with elevation in more open coffee forests (Table 2 and Fig 2b). Yet, only 3.6% of the variations in the total preliminary quality was explained by the model. The conditional R^2 (40.2%) indeed indicates a considerable spatial random variability for total preliminary quality.

On the other hand, neither the interactive effects of elevation and canopy cover nor the canopy cover alone had relationship with total specialty quality, caffeine, trigonelline and total chlorogenic acids. Positive relationships were observed between elevation and total specialty quality (explaining 3.8% of the variance), caffeine (explaining 6.1% of the variance), trigonelline (explaining 9.5% of the variance) and total chlorogenic acids (explaining 7.5% of the variance) and this variability is probably due to the climatic variables associated with elevation (Table 2 and Fig 2c and Fig 3). The low marginal R^2 values and relatively high conditional R^2 values again imply a strong random "farm" effect in our data.

A study conducted in southwest Ethiopia demonstrated that coffee trees grown under open conditions at 1780 m elevation produced lower quality beans, particularly with respect to acidity, body, and flavor compared to coffee from plants grown under dense shade (Bote 2016). However, this study was

conducted under an artificially developed shade net. A reduced shade level at lower elevations was found to have a negative effect on total preliminary quality. Avelino et al. (2007) found a positive relationship between increasing elevations (lower temperature) and quality for Arabica coffee cultivated under intermediate shade levels in Central America. A study in Uganda (Sarmiento-Soler et al. 2020) revealed that higher temperatures and vapor pressure deficit (increased transpiration) at lower elevations led to sub-optimum growing conditions for coffee. Studies conducted in southwest Ethiopia indicated that coffee beans grown at mid-elevation with less shaded conditions accumulated higher content of total chlorogenic acid. Based on their reports, the highest caffeine content was obtained from mid-elevation at dense shade (Tolessa et al. 2017).

Table 2: Overview of linear mixed effect model testing of elevation, canopy cover and management intensity on hundred bean mass, total preliminary and specialty quality and biochemical composition. The *p*-values are two-tailed at 0.05 from the linear mixed-effect model, Marg. R^2 is the proportion of variance explained by the fixed effects only (i.e. elevation, canopy cover and management) whereas Cond. R^2 is the proportion of the variance explained by both fixed and random effects (the whole model), both Marg and Cond R^2 values were given only for the full models. Management intensity refers to the extent of farm management among the coffee farms.

Response	Effect	Effect estimate	F (*p*-value)	Explained variance (%)	
				Marg. R^2	Cond. R^2
Hundred bean mass	Elevation	0.01	31.4 (**<0.001**)		
	Canopy cover	0.03	14.3 (**<0.001**)		
	Elevation x Canopy cover	-0.006	15.9 (**<0.001**)	27.9	30.2
	Management Intensity	-0.068	0.04 (0.847)		
	Intercept	-11.4			
Total preliminary quality	Elevation	0.006	1.4 (0.185)		
	Canopy cover	-0.03	5.4 (**0.014**)		
	Elevation x Canopy cover	0.002	4.9 (**0.019**)	3.6	40.2
	Management Intensity	1.262	1.18 (0.282)		
	Intercept	93.58			
Total specialty quality	Elevation	0.004	3.6 (**0.014**)		
	Canopy cover	0.05	0.6 (0.409)		
	Elevation x Canopy cover	-0.005	0.8 (0.342)	3.8	25.7
	Management Intensity	-0.16	0.21 (0.646)		
	Intercept	78.21			
Caffeine	Elevation	0.001	4.5 (**0.044**)		
	Canopy cover	0.02	1.6 (0.216)		
	Elevation x Canopy cover	-0.003	1.8 (0.177)	6.1	67.6
	Management Intensity	0.039	0.138 (0.71)		
	Intercept	-0.566			
Trigonelline	Elevation	0.001	3.62 (**0.023**)		
	Canopy cover	0.02	0.34 (0.238)		
	Elevation x Canopy cover	-0.006	0.51 (0.479)	9.5	68.5
	Management Intensity	-0.24	0.04 (0.857)		
	Intercept	-0.51			
Chlorogenic acids	Elevation	0.003	1.3 (**0.024**)		
	Canopy cover	-0.002	0.001 (0.323)		
	Elevation x Canopy cover	-0.002	0.005 (0.944)	7.5	61.6
	Management Intensity	-0.096	0.032 (0.860)		
	Intercept	1.944			

On the other hand, the lower bean mass at higher elevation for dense shade cover might be due to cooler temperatures (14.8°C – 16.7°C) and low incoming radiation, resulting in reduced coffee berry growth (although these are influenced by many other climatic variables like light intensity, light quality, sunshine hours, cloud cover, mist, etc.). Hence, a balanced shade canopy cover provides a means to keep coffee plants closer to their optimum temperatures ranges (18˚C – 21˚C) and prevent damage from extreme minimum and maximum temperatures (Lin 2006).

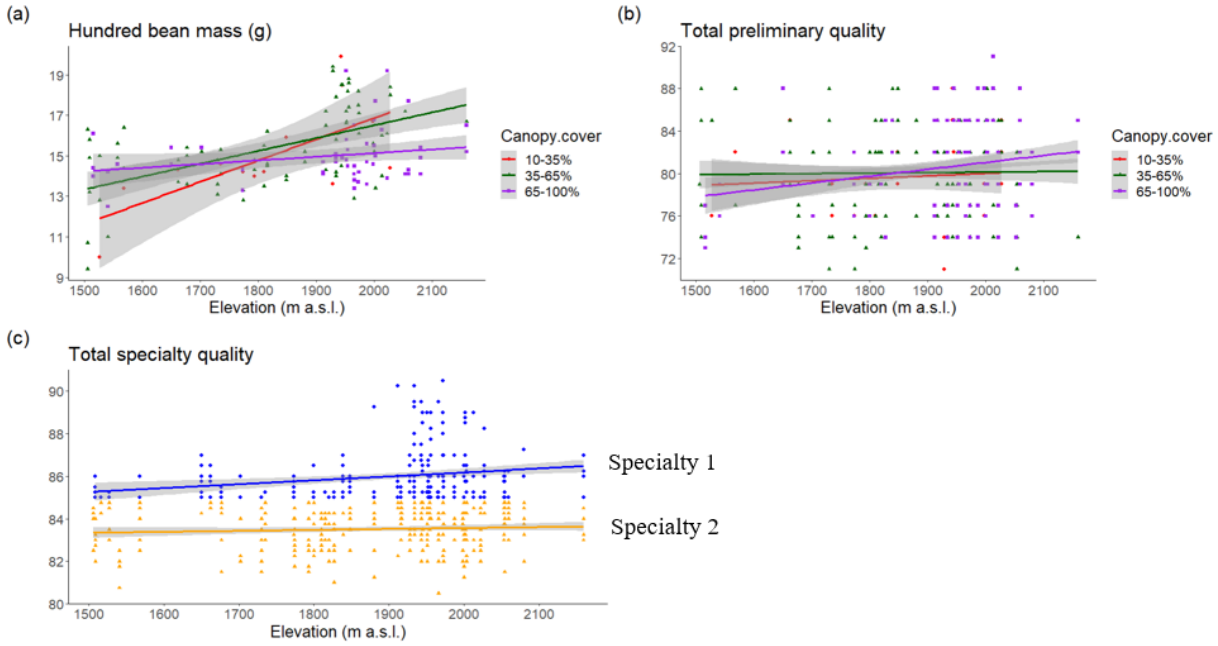

Fig 2. The relationship between elevation and shade canopy cover with (a) hundred bean mass, (b) total preliminary quality, and (c) total specialty quality. Data points represent a particular response variable at a single coffee tree (n=159) in which the fitted regression lines and 95% confidence intervals are from linear mixed-effect models at $p<0.05$.

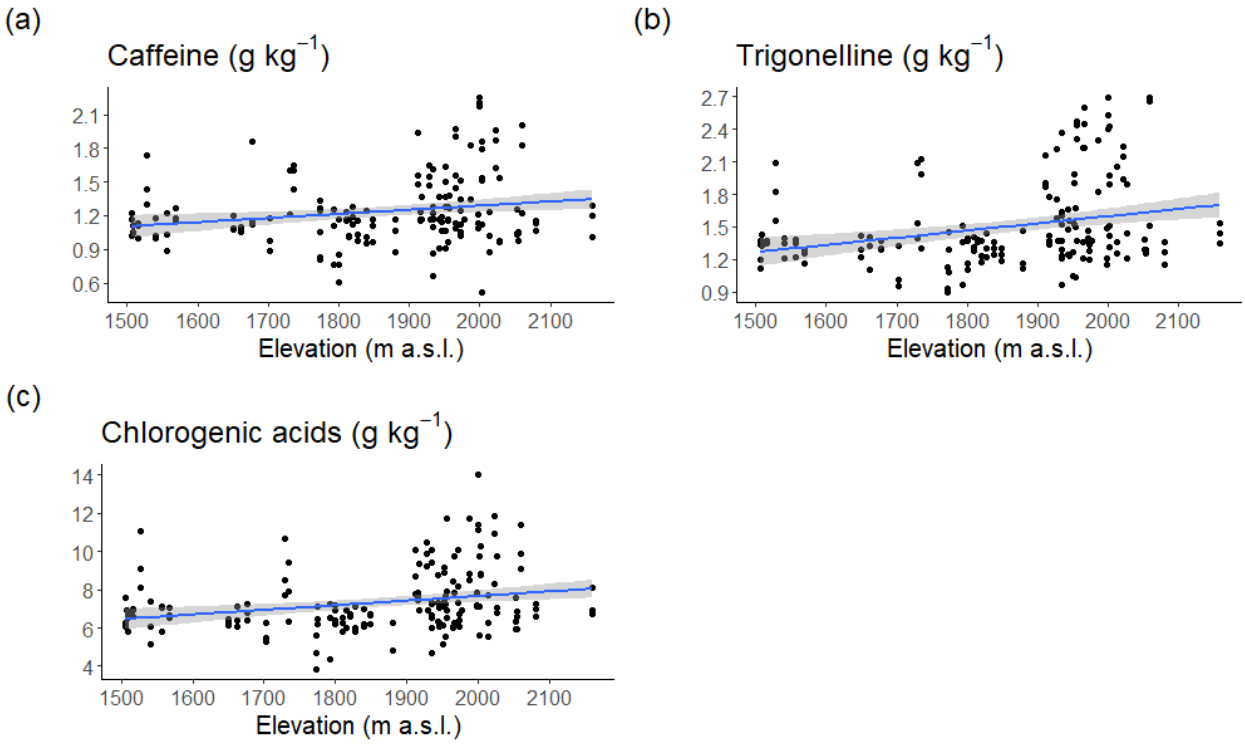

Fig 3. The relationship between elevation and (a) caffeine (b) trigonelline, and (c) chlorogenic acids content of green coffee beans. Data points represent a particular response variable at a single coffee tree (n=159) in which the fitted regression lines and 95% confidence intervals are from linear mixed-effect models at $p<0.05$.

Relationship between soil temperatures, chemical variables and bean quality and biochemistry

In the biplot where temperatures and soil chemical variables were condensed into PCA axes, the length of the vector indicates the variance explained by the variable. The angle between the vectors lines corresponds to the degree of association between the variables. The chemical soil variables that clustered to the right (soil Ca, Mg and C, K-CEC and CEC-K) have large positive loadings, i.e, the relationship among these variables is strong and positive. A strong positive relation was observed between soil N and soil C/N. Likewise, a positive relationship was observed between soil pH and Olsen-P (Appendix Fig S2). On the other hand, the soil temperature variables cluster to the right, minimum and maximum temperatures, have large positive loadings and are strongly correlated with each other (Appendix Fig. S1).

Soil chemical variables were found to have a significantly positive relationship with hundred bean mass whereas a significantly negative relationship was observed with soil temperatures, in which 14.7% of the variation in hundred bean mass could be explained by soil chemical variables and soil temperatures together (Table 3). Similarly, the total preliminary quality was found to be correlated significantly with soil temperatures both positively (PC1) and negatively (PC2) while it had a significant positive relationship with soil chemical variables. 71% of the variation could be explained by soil chemical variables and soil temperatures (Table 3 and fig 4). Total specialty quality showed a significantly positive relationship with soil properties, in which 84% of the variation could be explained by soil chemical variables (Table 3). Soil temperatures were found to have a strong and significant negative relationship with caffeine, trigonelline and total CGA (Table 3 and fig 4). On the other hand, soil chemical variables were found to have a significantly positive relationship with caffeine, trigonelline and total CGA (Table 3 and fig 4).

As part of the tropical climatic belt, southwestern Ethiopia is characterized by a reduced seasonal temperature variation, with elevation being the major driving factor of temperature at larger spatial scales. At the local scale, shade tree canopy cover is the main determinant of local microclimate temperature in this system. During the course of this study, an attempt was made to measure air temperature but due to theft it was impossible to get the loggers in the field. However, literature relating soil temperatures with coffee bean quality and biochemistry are rarely available and hence, our main focus was given to measure this variable. Meanwhile, soil temperature is an important environmental variable but it is a rarely reported indicator of climate change. Based on previous reports, soil temperature was found to be equally important with the local air temperature under shaded environment in explaining tree performance. The findings showed that there is a strong correlation between local air temperature and soil temperature at different depths. The annual and seasonal mean local air and soil temperatures mainly displayed significant trends of increase over years, although the rise of the mean air and soil temperatures was a bit asymmetric (Leeper et al. 2021; Zhan et al. 2019). Shade tree canopy

cover modulates macroclimatic trends through effects on local microclimates. In this regard, dense tree canopies not only lower ground-layer temperatures but also increase relative humidity and shade in the understory. Higher relative humidity in dense shaded environments can also protect trees underneath from drought. All together it can buffer the impacts of regional climate change effects (De Frenne et al. 2013; Von Arx et al. 2013). Our present study confirmed that high-quality coffee beans were mostly produced at high elevations (cool temperatures). Temperature during fruit development period (flowering to fruit maturity) is a crucial driving variable influencing brew quality and green bean biochemistry. In the present study, cool climates (i.e., more elevated conditions and dense canopy cover) had a high potential to produce coffee beans having superior total preliminary quality, higher caffeine, total CGA contents, and trigonelline concentrations as compared to coffee growing in warmer microclimates (low elevations with light shade).

As already demonstrated by the PCA, the total preliminary quality is strongly and significantly correlated with temperature (in terms of effect size and beta-values), where 72.3% of the variations are explained by temperature and soil chemical variables. This implies that the share of farm variability other than soil temperature and soil chemical variables tend to be relatively small as demonstrated from the marginal and conditional R^2 values (Table 3 and Fig 4). The results further support that coffee cup quality attributes are sensitive to soil temperature changes and soil chemical properties. However, there are many climatic variables that were not measured which might have effects on cup quality. The study of Bertrand et al. (2012) demonstrated that, among the climatic factors, mean temperature during coffee seed development greatly influenced the sensory profile and cup quality attributes such as acidity, fruity character and flavor were obtained from the cool climates.

Coffee does not tolerate a wide range of temperatures in that mean temperatures below 16°C and above 23°C are suboptimal and the optimum temperature for Arabica coffee growth ranges between 18°C to 21°C (Camargo 2010; Kirkpatrick 1936). In our present study, the minimum, mean and maximum soil temperatures during the coffee fruit development period (flowering to fruit maturity) ranges between 9.5°C – 17.5°C, 14.5°C – 21.0°C, and 17.5°C – 36.5°C, respectively, about 1.5°C cooler than the lower limit (16°C) for coffee Arabica. In our network of coffee plots, we did not detect an apparent effect of management intensity on the studied coffee quality attributes.

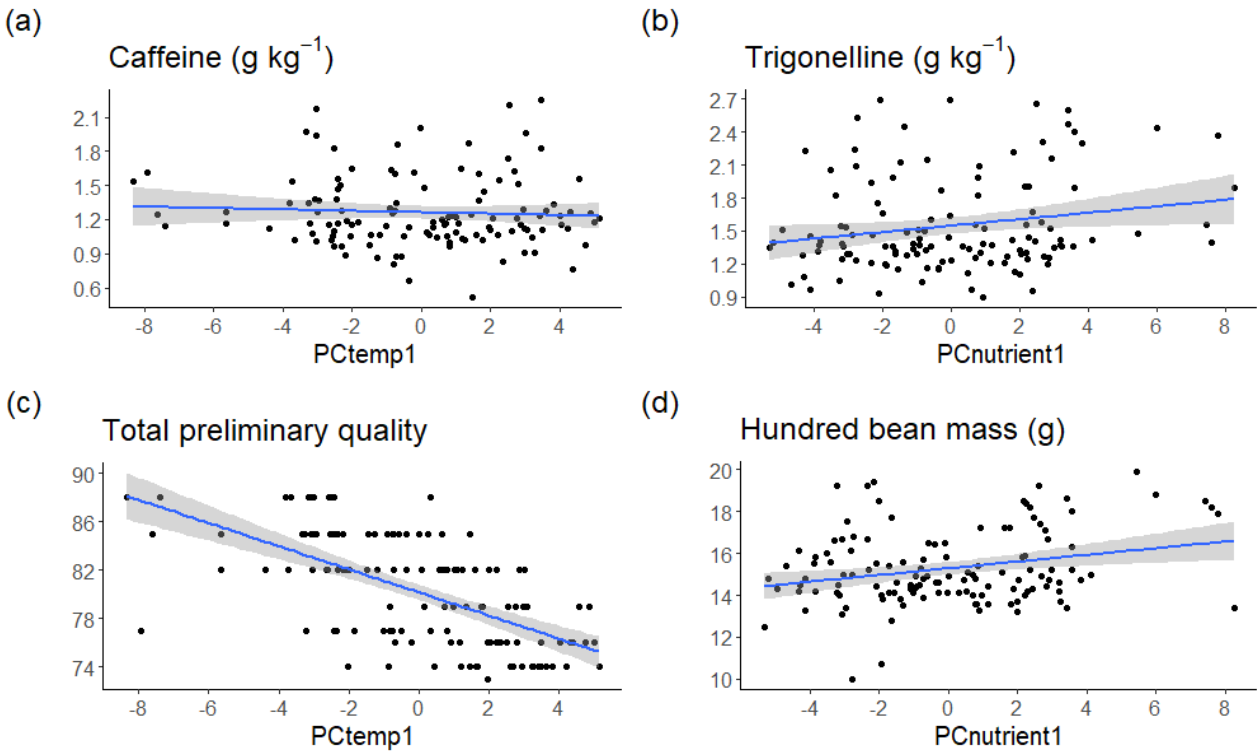

Fig 4. Scatterplots of (a) caffeine, (b) trigonelline, (c) total preliminary quality and (d) hundred bean mass as a function of soil nutrients (PCnutrient1) and soil temperatures (PCtemp1) principal components. Data points represent a particular response variable at a single coffee tree (n=159) in which the fitted regression lines and 95% confidence intervals are from linear mixed-effect models at $p<0.05$. The principal component biplots are included in supplementary information.

On the other hand, a hundred bean mass showed both positive and negative relationships with temperature and only 14.3% of the variations are explained by both temperature and soil chemical variables (Table 3). This implies that temperature could have both positive and negative associations with hundred bean mass and the magnitude of this relationships on coffee quality attributes depend more on other farm variables other than what temperature and soil chemical variables explained. Our findings tend to show that dense shading conditions reduced hundred bean mass at very high elevations. In contrast, dense shading at warmer (lower elevations) increased bean mass (Fig 2).

As indicated in Vaast et al. (2006) and Laderach et al. (2017), the faster fruit development due to elevated temperature results in malformed beans with poorer cup quality, which is the result of the consequence of excessive demand for resources from the seed endosperm in a compressed timeframe. According to a report of Ramalho et al. (2018), prevalence of high temperatures in the field conditions often coincides with decreased water availability and high atmospheric vapor pressure deficit, resulting in lower coffee bean quality because high VPD causes water limited conditions which in turn can worsen the stress effects on coffee plants by increasing the transpiration rate or by reducing carbon uptake (Avila et al. 2020, Xu et al. 2016; Belko et al. 2012). The findings of Silva et al. (2005), showed

that temperature was likely the most important factor to bring variations in cup quality and bean biochemistry of coffee from the southwest region of Ethiopia.

Green bean biochemical compositions (caffeine, trigonelline and total CGA) were negatively correlated with temperature, and around 45% of the variation in the data was explained by temperature and soil chemical variables. However, the share of random farm variability other than temperature and soil chemical variables was still large (roughly 20% of the variation in the data) (Table 3). The results further support that green bean biochemical compositions are very sensitive to temperature changes and soil chemical variables. Caffeine, trigonelline and total CGA were found to be favored by shading (Farah et al. 2006; Somporn et al. 2012), whereas other studies obtained contradictory findings, for instance, total CGA was favored by open sun (Tolessa et al. 2017; Somporn et al. 2012; Vaast et al. 2006). Some other studies reported a negligible correlation between total CGA content and shading (Avelino et al. 2007). The effect of shade canopy cover and elevation on soil temperatures and chemistry was reported in an earlier study (Getachew et al. 2022). We then found that the interactive effect of elevation and shade tree canopy cover had a significant and strong effect on mean soil temperatures. On the other hand, Olsen-P decreased with increasing elevation. Contrarily, soil C/N increased with increasing shade canopy cover (Getachew et al. 2022). Although we here do not find a direct link between shade canopy cover and bean biochemistry, shading had a significant indirect influence on bean biochemistry through modification of the local microclimatic conditions and soil chemical variables (Fig 3, Table 3). This confirms our expectations that increased light and warming below a more open canopy can negatively influence the synthesis of various bean quality precursors (such as caffeine, trigonelline and total CGA) by accelerating berry maturation and disfavoring bean-filling and brew quality (Bertrand et al. 2012, Joet et al. 2010, Geromel et al. 2008, Vaast et al. 2006).

Caffeine in green beans co-determines flavor and the quality of the final coffee beverage. Caffeine is associated with the bitterness and flavor of the coffee. The caffeine content in the green coffee beans showed a great variability with values ranging from 0.5 to 2.3 g per 100 g. Trigonelline contents obtained in this study showed a large variability too with values ranging from 0.9 to 2.7 g per 100 g. Derivatives of trigonelline are known to be an important precursor of the volatile compounds that contribute to the aroma and taste of roasted coffee (Cheng et al. 2016; Joet et al. 2010; Malta and Chagas 2009; De Castro and Marraccini 2006). Indeed, most studies reported a decreasing quality with decreasing trigonelline levels. Green coffee beans of arabica genotypes possesses chlorogenic acid contents that range between 3.5 – 7.5 g per 100 g (Gloess et al. 2013) while the total CGA content obtained in our present study ranged from 3.8 to 14 g 100 g. Total CGA contents is related with the astringency and bitterness in coffee brews (Gloess et al. 2013; Farah et al. 2006) and the content showed an increasing trend with increasing brew quality. Although most literatures have often associated high levels of CGA with lower brew quality (Farah et al. 2006; De Castro and Marraccini 2006), suggesting

that it is possible to obtain coffee brews with various levels of total CGA. Barbosa et al. (2019); Corso et al. (2016); Dos Santos Scholz et al. (2016); Zanin et al. (2016) and Kitzberger et al. (2013) also reported that good cup quality roasted coffee beans could also show wide variability in their CGA contents. Possible explanations for this difference could include the genetic differences of the Arabica genotypes, coffee tree age variations; time and temperature of bean roasting, and brew preparation processes.

Table 3. Effects of soil temperature and chemical variables on hundred bean mass, total preliminary and specialty quality and biochemistry of green coffee beans. Marg. R^2 is the proportion of variance explained by the four PC's used as predictors; whereas Cond. R^2 is the proportion of the variance explained by both the PC's used as predictors and the random effect (full model), both Marg. and Cond. R^2 values were given only for the full models. PCnutrient1 = the 1st principal component of soil chemical variables; PCnutrient2 = the 2nd principal component of soil chemical variables; PCtemp1= the 1st principal component of soil temperatures; PCtemp2= the 2nd principal component of soil temperatures.

Response	Predictors	Effect estimate	Std. Err	Standardized regression coefficient	*P-value*	Explained variance (%)	
						Marg. R^2	Cond. R^2
Hundred bean mass (g)	PCtemp1	4.02	1.19	3.35	0.0012	14.7	28.7
	PCtemp2	-0.41	0.14	-2.89	0.005		
	PCnutrient1	4.29	1.36	3.95	< 0.001		
	Intercept	14.22					
Total preliminary quality	PCnutrient2	0.321	0.112	3.323	0.020	71.1	85.2
	PCtemp1	-1.048	0.068	-15.322	< 0.001		
	PCtemp2	1.709	0.129	13.175	< 0.001		
	Intercept	84.26					
Total specialty quality	PCnutrient1	0.52	0.008	70.52	< 0.001	83.5	88.5
	PCnutrient2	0.29	0.02	13.25	< 0.001		
	Intercept	88.65					
Caffeine (g kg^{-1})	PCtemp1	-1.30	0.19	-6.88	< 0.001	45.8	68.5
	PCtemp2	-0.19	0.02	-8.47	< 0.001		
	PCnutrient1	2.13	0.26	7.74	< 0.001		
	PCnutrient2	0.09	0.01	7.05	< 0.001		
	Intercept	1.29					
Trigonelline (g kg^{-1})	PCtemp1	-1.67	0.26	-6.39	< 0.001	42.7	68.23
	PCtemp2	-0.21	0.03	-7.01	< 0.001		
	PCnutrient1	1.69	0.26	6.31	< 0.001		
	PCnutrient2	0.15	0.02	6.67	< 0.001		
	Intercept	1.54					

Chlorogenic acids (g kg^{-1})	PCtemp1	-7.12	1.07	-6.66	< 0.001	47.2	65.7
	PCtemp2	-0.91	0.12	-7.33	< 0.001		
	PCnutrient1	7.01	1.07	6.53	< 0.001		
	PCnutrient2	0.65	0.08	8.49	< 0.001		
	Intercept	4.52					

The present study showed that soil chemical properties are positively associated with hundred bean mass, green bean quality, and biochemical composition. This implies that fertile soils produce heavier, high quality beans with higher levels of caffeine, trigonelline and chlorogenic acids. Interestingly, selected soil chemical properties are strongly correlated with the total specialty quality (Table 3 and Fig 4). The measured soil chemical variables explained 84% of the variations in total specialty quality. This suggests that soil chemical fertility is decisive for improving total specialty quality. Hence, we can maximize specialty 1 (Q1) by growing coffee at higher elevations, while sustaining soil fertility. According to Yadessa et al. (2020), soil fertility parameters considerably affected the cup quality of wild Arabica coffee in its natural habitat. The study indicated that coffee with improved cup quality was collected from coffee farms having greater available P, K, Mg, and Zn levels. The compounds are considered important for the brew quality and also N and K certainly played a significant role in the final bean quality (Clemente et al. 2015).

Low temperature at high elevated areas retarded root and microbial activity and thus less phosphatase production compared with lower elevated sites. Besides, water-soluble phosphorus decreased with the decreased soil temperature due to the reduced movement of phosphorus in the soil controlled by diffusion (Onwuka and Mang 2018). As both the roots and microorganisms synthesize and release phosphatases to soil, soil available P might be reduced at higher elevations. However, higher levels of available P content in lower elevations had no contribution in improving the brew quality and green bean biochemistry, suggesting that the indirect relationships due to the natural soil P variation and the possible effects of P-fixation in the soil might render soil P less available for optimal coffee plant growth and fruit quality and composition (Rekik et al. 2019).

The novelty of this study lies in the investigation of soil temperatures on coffee quality and bean biochemical compositions in that intermediate to deep shade moderates responses to the increasing temperatures at medium and higher elevations and improved coffee bean quality. Hence, we can conclude that coffee beans from cool climates (higher elevations) would have a greater bean quality and better bean biochemical compositions which in turn will have a better commercial value. The main weakness of this particular study is the missing data particularly on essential climatic variables such as ambient air temperature, relative humidity, vapor pressure deficit, soil moisture, solar radiation, and sunshine hours.

Conclusion and implication for management

We found that the highest cup scores (better quality coffee) are produced at higher elevations with the highest concentrations of caffeine, chlorogenic acids and trigonelline. It seems that coffee quality from farms at lower elevations (mainly below 1600 m) will suffer most from changing climatic conditions for the areas under study (southwest Ethiopia). Besides, fertile soils produce heavier beans, beans possessing good brew quality and green bean biochemistry. Hence, we can conclude that coffee beans from cool climates and good fertile soils would have a better commercial value. Spatial random variability at the farm level is an important consideration in our studied coffee bean attributes. Hence, future studies need to consider coffee tree genetic differences, coffee tree age and essential climatic variables such as ambient air temperature, relative humidity, vapor pressure deficit, soil moisture, solar radiation, and sunshine hours for a better understanding of brew quality and green bean biochemistry. In addition, shade tree species and the genetic variation of the coffee trees should not be forgotten.

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Code availability: Not applicable.

Authors' contribution: Conceptualization, M.G., P.D.F., K.V. and P.B.; methodology, M.G., K.T., K.V., K.H., A.T., B.A. and P.B.; formal analysis, M.G.; investigation, M.G. and K.T., writing—original draft, M.G. and P.D.F.; writing—review and editing, P.B., K.H., A.T., and K.V.; visualization, M.G., and B.A.; Funding Acquisition, P.B.; Supervision, K.V., P.B. and P.D.F.

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Data availability: The datasets analyzed during the current study are available from the corresponding author on reasonable request. Moreover, the data will be made available after publication via an online repository such as figshare.

Declarations

Conflict of interest: The authors declare no potential conflict of interest.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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