Shade tree species effects on soil biogeochemistry and coffee bean quality in plantation coffee agroforestry

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- 15 Abstract

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Shade trees are used in many coffee production systems across the globe. Beyond the benefits 16 17 on biodiversity conservation, climate buffering, carbon sequestration and pathogen regulation, 18 shade trees can impact the soil nutrient status via, for instance, litter inputs and nitrogen fixation. Since soil nutrients affect coffee quality and taste, there is also a potential indirect 19 effect of shade tree species on coffee quality. Yet, in spite of the potentially large impact of 20 shade tree species, quantitative data on the effects of shade trees on (i) soil biogeochemistry 21 and (ii) the associated coffee bean quality remain scarce. We quantified to what extent four 22 widely used shade trees species (Acacia abyssinica, Albizia gummifera, Cordia Africana and 23 Croton macrostachyus) in a plantation coffee agroforestry system in Ethiopia impact soil 24 biogeochemistry, and how this in turn affects coffee quality, measured as cupping scores. We 25 found especially significant negative impacts of N-fixing shade tree species on soil pH and 26 base cation concentrations. Plant-available and total phosphorus was enhanced by the presence 27 of Albizia gummifera. Thus, the present findings demonstrate that careful selection and 28 integration of shade tree species such as Acacia abyssinica and Albizia gummifera into coffee 29 production systems is a good practice for sustaining soil chemical properties in coffee 30 agroecosystem. In spite of the impacts on soil characteristics, the shade tree species did not 31 impact cupping scores of the resulting coffee beverage except some effect on the bean mass. 32 Hence, further research should focus more on coffee-shade tree associations such that our 33 understanding of the biogeochemical impacts can be improved, especially given the 34 microclimatic importance of shade tree species in buffering the negative impacts from 35 36 heatwaves and droughts due to climate change.

38 Keywords: Coffee quality, soil biogeochemistry, shade tree species identity, Ethiopia

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1. Introduction

Including shade trees in annual or perennial cropping systems has been promoted as a potential
solution to bridge soil conservation efforts and improve crop yield in many tropical countries
(Montagnini *et al.* 2017; Tscharntke *et al.* 2011). The positive effect of including shade trees
with coffee include microclimate buffering against heatwaves and drought (Getachew *et al.*2022; Merle *et al.* 2022), carbon sequestration (Soils *et al.* 2020; Dhyani, 2017; Jose and
Bardhan, 2012), and improved biodiversity conservation and soil fertility (Tscharntke *et al.*2011).

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Shade trees can impact the soil nutrient status both directly (e.g. via litter inputs and nitrogen 49 fixation), but also indirectly (e.g. via altered decomposition rates due to contrasting below-50 canopy temperatures) (Liu et al. 2021; Strukelj et al. 2021). The presence of shade tree species 51 at the ecosystem level can improve resource use complementarity (Mahaut et al. 2020), 52 minimize nutrient leaching (Cappelli et al. 2022), and improve nutrient recycling and nutrient 53 availability for the crops (Muchane et al. 2020; Sileshi et al. 2020; Kuyah et al. 2019). These 54 benefits would be predominantly important for coffee cultivation practiced with no/little 55 56 external inputs, which tend to be nutrient-depleted systems because nutrients exported from the system may not be replaced through fertilization (Nzeyimana et al. 2016). Besides, by 57 harvesting coffee, nutrients are also exported from the system, and unless external inputs are 58 added, several nutrients can become limiting (Kiup et al. 2017). 59

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N-fixing shade trees, due to symbiosis with root-nodulating bacteria, can significantly 61 62 influence the soil nutrient status due to their N-fixing capability and effects on soil pH (Sileshi et al. 2020; Franklin et al. 2019; Hedin et al. 2009). Higher N content in litter favors microbial 63 64 activity and diversity, which in turn increase N cycling (Braga et al. 2019; Lopez-Sampson et al. 2020). The provision of a sustainable N cycling in agroforestry systems is hence the result 65 of these dynamics as it reduces N leaching (Karki et al. 2021). A linear decrease in N loss with 66 increasing shade tree cover was shown in coffee cultivation (Tully et al. 2012). Similarly, shade 67 68 tree species enhanced the organic matter concentrations due to litter inputs, thereby potentially favoring microbial diversity (Soils et al. 2020; Velmourougane, 2017; Bagyaray et al. 2015). 69 70 Shade tree species may have varying effects on soil pH. N-fixing trees have been shown to reduce soil acidity as compared to non-N-fixing shade trees (Muchane et al. 2020; Tully et al. 71

2013). Yet, Etafa (2022) reported an increased soil pH under *Acacia abyssinica* and *Albizia gummifera*. The density of soil bacteria is negatively affected by soil acidity (Neina, 2019;
Tully *et al.* 2012). Meanwhile, both high and low soil pH negatively affect plant nutrient availability and uptake (Bidalia *et al.* 2019).

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77 Because of changed soil nutrient status, litter inputs and soil acidity, also plant-available phosphorus (P) concentrations can be affected. P has been shown to be a limiting nutrient in 78 many coffee agroecosystems (Notaro et al. 2022; Soils et al. 2020; Notaro et al. 2014), and 79 80 hence the contribution of litter from shade trees is suggested to have a considerable positive effect on P cycling to enhance the available P content in soils (Aleixo et al. 2020; Notaro et al. 81 2014; Xavier et al. 2011). This is due to the higher degree of mycorrhizal symbiosis in the 82 presence of shade trees and also because of the different canopy exchange processes that are 83 strongly species-dependent (Zhang et al. 2022; Ekqvist, 2015). P-cycling in a coffee shade tree 84 85 mixed system depends on the specific characteristics such as location and management system (Sauvadet et al. 2020, Ekqvist, 2015). Aleixo et al. (2020) indicated that N-fixing shade trees 86 can have a positive effect on plant-available P as compared to fields with non-N-fixing shade 87 tree species. Soil pH is also related to plant available-P in the sense that at relatively higher pH, 88 89 plant available-P increases (non-linearly) (Buczko et al. 2018). On the other hand, in high alkaline soils, phosphorus reacts with calcium and also becomes inaccessible. 90

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Although the central goal of agroforestry is to create complementarity between shade trees and 92 93 coffee plants, growing coffee in association with shade trees inevitably leads to some degree of competition for the aboveground (light) and belowground resources (water and nutrients) 94 (Sebuliba et al. 2022; Schwendenmann et al 2010; Lin et al. 2008). Different shade tree species 95 bring alterations in light, soil water content and nutrient competition with the coffee plants due 96 97 to variations in canopy and root architecture. For instance, Campanha (2004), reported that shade trees used part of the available nutrients for growth and development, while Sebuliba et 98 al. (2022) and Siles et al. (2010) reported that shade trees competed with coffee for soil 99 nutrients and soil moisture. However, the degree to which this occurs will be largely controlled 100 through appropriate selection of the shade tree species and management if microclimatic 101 protection and nutrient cycling are the main goals. 102

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104 Soil pH and the nutrient status can also impact coffee bean quality. Body, acidity and cup 105 cleanness are directly correlated with soil pH (Morales-Ramos *et al.* 2020; Castro-Tanzi *et al.*

2012). Excess N increases the caffeine content, resulting in a more bitter taste of the brew 106 (Koehler, 2017; Petit, 2007). A recent study from southwest Ethiopia revealed that soil fertility 107 variables affected coffee bean physical and cup quality in its natural habitat and shows that 108 available P, K and the ratio between Mg and K were the most important soil chemical variables 109 influencing coffee bean size (Yadessa et al. 2020), and cupping scores and green bean 110 biochemistry (Getachew et al. 2022; Clemente et al. 2015). Excessive NPK fertilizer use and 111 increased aluminum toxicity were shown to cause a reduction in coffee cup quality due to a 112 reduced soil pH (Rekik et al. 2019; Castro-Tanzi et al. 2012). Another study shows that coffee 113 114 cup quality was significantly associated with available P and K (Yadessa et al. 2020). To date, research linking soil biogeochemistry and coffee bean quality is, however, very scarce. 115

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Although several studies conducted in the tropics have quantified soil nutrient responses to 117 shade tree species, quantitative data on individual nutrients, and linked with data on coffee 118 quality, under different shade tree species remains limited. Hence, the objective of this study 119 was to test the hypothesis, derived from the above studies, that shade tree species impact soil 120 121 biogeochemistry and in turn could affect coffee bean quality. We here thus test whether and how shade tree species in plantation agroforestry systems impact soil biogeochemistry, and 122 123 how this could be related to changes in coffee bean quality. To avoid effects of pre-existing soil nutrient differences, we used a common garden approach in which coffee was grown below 124 125 shade trees of different species within one large coffee plantation.

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127 **3.** Materials and Methods

128 **3.1. Study area and experimental design**

129 The study was carried out at a commercial coffee plantation located on a flat plateau in the eastern wing of Horizon-2 coffee farm in the southwest region of Ethiopia. The locations where 130 131 the coffee plants were sampled lie at a geographical coordinate ranging between 7.94 - 07.95 N latitude and 36.63 - 36.64 E longitudes (Fig. 1), with the elevation ranging between 1545 -132 1570 m asl. The region is part of the Eastern Afromontane Biodiversity Hotspot (Hundera et 133 al. 2013) where the climate is conditioned by the Inter-Tropical Convergence Zone (Schmidt 134 135 et al. 2014), with a yearly rainfall between 1500 and 2000 mm. Besides, the region is characterized by a unimodal rainfall, accounting for about 85% of the annual rainfall, with the 136 137 main rainy season between May and September with the main dry season between December and March. Differences in temperature vary throughout the year with a mean monthly 138

temperature between 13 and 26°C (Geeraert et al. 2019; Denu et al. 2016). The bulk of coffee 139 growing soils in the region are classified as Eutric Nitisols, which are deep, red and well-140 drained soils with a clay content of more than 30% and a pH (measured in H₂O) between 4.2 141 and 6.2 (Kebede et al. 2018; Kufa, 2011). In terms of farming systems, the southwestern region 142 of the country is characterized by a mosaic of farmlands dominated mainly by Arabica coffee 143 farming systems. The region is known as a primary center of origin and diversity of coffee, 144 where the species grows naturally as understory tree in moist Afromontane forests (Hundera et 145 al. 2013; Davis et al. 2012). Coffee in this region is mainly grown under shade trees, either 146 147 within forest or forest-like environments, or in farming systems that deliberately incorporate specific shade trees. Common coffee shade tree species in this region include Albizia 148 gummifera, Acacia abyssinica, Cordia africana, Croton macrostachyus, and Millettia 149 *ferruginea*. The intensively managed commercial coffee plantation is run by a private company 150 in which regular field management activities including pruning, weeding (herbicide use) and 151 152 fertilization are a regular practice.

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154 Selection of individual shade tree species

In March 2020, four shade tree species were selected across the commercial plantation to study
effects of these widely used shade tree species on soil biogeochemistry and coffee bean quality:
two N-fixing shade tree species (*Acacia abyssinica* and *Albizia gummifera*), and two non-Nfixing shade tree species (*Cordia africana* and *Croton macrostachyus*).

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Acacia abyssinica is N-fixing tree in the family Fabaceae. The tree is found in Africa from east 160 to south. In Ethiopia it occurs in wooded grassland, highland forest edges of dry, moist and wet 161 162 highlands of the agroclimatic zones. Its main uses among others are for firewood, charcoal, poles, fodder, bee forage, shade tree for coffee, soil conservation, and fence. Albizia gummifera 163 164 (also Fabaceae) is commonly grown in lowland and upland rainforest, riverine forest, and in open habitats near forests. Its uses among others are shade tree for coffee (in Ethiopia), 165 166 fuelwood, timber, and erosion control. Cordia africana occurs at medium to low elevations, in woodland, savannah, in warm and moist areas. It occurs in afro-montane rainforest along 167 margins and in clearings. It provides good bee forage, as the flowers yield plenty of nectar. 168 Beehives are often placed in this tree and also is planted as a shade tree in coffee plantations. 169 170 It is usually left in the fields, as it provides excellent shade for crops and a good source of firewood and Timber. 171



Fig 1. Study area showing an aerial view of the study landscape representing the distribution of shade
tree species (28 locations).

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Croton macrostachyus (Euphorbiaceae) is widespread in areas with high rainfall. It is 176 commonly planted for shade and provides good bee forage, as the flowers yield plenty of 177 nectars. It also used for firewood, shade for crops, mulch, and soil conservation (Teketay and 178 Tegineh, 1991). Each shade tree species was replicated seven times and a total of 28 shade tree 179 180 species identity (from 28 locations) were considered. The canopies of selected shade trees were not overlapping with other tree canopies, and the selected shade trees were spaced at least 30 181 m from each other, and were also spatially intermixed to avoid spatial autocorrelation between 182 trees belonging to the same species (Fig. 1). From each of these shade trees, three coffee plants 183 that are completely positioned under the canopy of the shade trees were considered (Ayalew et 184 al. 2022). 185

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Characteristics of the commercial coffee plantation 188 189

The plantation commonly applies a compound NPK fertilizer (18-18-24) based on the 190 productivity of the individual coffee trees/plots/year. The commercial fertilizers are mostly 191 applied in spray forms and the application modality in the coffee farms is: NPK (75 g/tree/year) 192 applied in two splits and urea (28 kg/ha) applied as a foliar spray once per year as a 193 supplementary feed also to reduce the potential of leaching losses. On a hectare basis, this 194 would be 250 kg NPK/ha (considering 1.5m between plants and 2m between rows in a coffee 195 plantation). Herbicides are commonly used to control weeds, and fungicides are also applied 196 once per year on a plant basis based on the incidence of fungal disease. 197

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199 **Coffee plant-level measurements**

Three randomly selected coffee plants that are completely positioned inside the canopy of the 201 shade trees were marked for measurements. A total of 84 coffee plants were marked from the 202 28 shade trees (28 locations; Fig. 1) and all of the required samples (soil sampling, canopy 203 cover measurements, and coffee cherry sampling) were taken from each of the coffee plants. 204 205 The following sub-sections describe the data collected at a coffee plant level.

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Measuring shade tree canopy cover: Shade tree canopy cover over each coffee tree was 207 quantified using a convex spherical crown densiometer (Forest densiometers, Model A, 208 209 Bartlesville, Oklahoma, USA). The densiometer is made of a small wooden box with a convex mirror consisting of a grid of squares; shade tree canopy cover is then calculated as the 210 211 proportion of 96 points that was intersected by vegetation times 1.04. The densiometer was held at breast height and the observer's head was reflected from the edge of the mirror just 212 213 outside the box. The curved mirror reflects the canopy above. Above the canopy of each sampled tree using a ladder all the time, two counts were recorded and their average was taken. 214 215

Measuring soil biogeochemistry 216

A cylindrical metal core sampler 5 cm in diameter and 15 cm long was used to sample the 217 undisturbed soils in March 2020. The core was driven to the desired depth (10 cm) surface 218 mineral topsoil (0-10 cm) and samples were taken from three locations per coffee tree (10 cm 219 away from the coffee stem in three cardinal directions). Then, the soil was carefully taken from 220 the core sampler to preserve the known soil volume in situ. In doing so, surface litter and plant 221 debris were carefully removed from the samples. The soil samples were taken during the 222

measurement of the canopy cover. These three field samples per shrub were pooled into one sample, immediately weighed and then oven-dried at 65° C for 48 hrs. Finally, the soil samples were sieved with a 2 mm mesh and stored for further soil nutrient analysis.

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Laboratory analysis of soil samples: The oven-dried soil samples were used for the 227 measurements of soil organic carbon, soil total N, soil pH (H₂O), Olsen-P, lactate-P, oxalate-228 P, total-P, lactate-K, lactate-Mg, lactate-Ca, lactate-Al, oxalate-Al, and oxalate-Fe. The pH (in 229 H₂O) of the soil was measured using a calibrated glass electrode (model Ross sure-flow 8172 230 231 BNWP, Thermo Scientific Orion, USA). Dried soil samples without further preparations were combusted with elemental analyzer to analyze total C and N content. The samples were 232 combusted at 1150°C and the gases were measured by a thermal conductivity detector in a CNS 233 elemental analyzer (vario Macro Cube, Elementar, Uberlingen, Germany). The CNS measures 234 the content of C and N inorganic soil samples. Soil available phosphorus was analyzed using 235 various extraction methods for a comparison of different methods. In so doing, three extraction 236 methods were used: Olsen, lactate and oxalate and also total-P was also analyzed. In all cases 237 238 P was analyzed colorimetrically with the malachite green procedure according to Laitha et al. (1999). For Olsen-P, sample extraction of 2.5 g dry soil with 50 ml 0.5 M sodium bicarbonate 239 240 (NaHCO₃) was conducted at pH 8.5. For lactate-P, samples were extracted in a 1:5 soil: extractant ratio with ammonium lactate which consisted of lactic acid (88%), acetic acid (99%) 241 and ammonium acetate (25%) at pH 3.74 according to the malachite green procedure (Lajtha 242 et al. 1999). For Oxalate-P, Oxalate-Al and Oxalate-Fe, Active P, which also includes P that 243 can become available on the longer term and is adsorbed by Al and Fe. This P-fraction was 244 extracted in ammonium oxalate-oxalic acid (according to NEN 5776:2006). P-contents were 245 measured according to the malachite green procedure. Al and Fe contents were measured by 246 atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) whereas total-P was 247 measured after complete destruction of the soil samples with HClO₄ (65%), HNO₃ (70%) and 248 H₂SO₄ (98%) in teflon bombs for 4 hrs at 150°C. P-contents were measured colorimetrically 249 according to the malachite green procedure (Lajtha et al. 1999). Lactate Ca, Mg, K, and Al, 250 was extracted in a 1:5 soil: extractant ratio with ammonium lactate which consisted of lactic 251 acid (88%), acetic acid (99%) and ammonium acetate (25%) at pH 3.74. The cations (Ca, K, 252 Mg, Al) were measured by atomic absorption spectrophotometry. 253

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- 256 Coffee berry sampling and coffee quality assessment
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All fully ripe, red-colored coffee berries were hand-picked once at peak harvest in November 258 2020 from each selected coffee tree using the local coffee bags ("Kesha"). The berries were 259 dry-processed, i.e. sun-dried (on raised mesh wire) immediately after harvest (harvesting was 260 261 in the morning and subjected to drying started in the afternoon). The berries were returned to local coffee bags "Kesha") before sunset and stored in clean rooms (to prevent spoilage) and 262 were exposed to the sun in the morning until green beans attained 11.5% moisture content 263 264 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 265 and packed for analysis. The dried coffee berries were dehusked using a hulling coffee machine 266 (coffee huller, McKinnon, Scotland) at Jimma University, cleaned and stored at room 267 temperature. 268

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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.

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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.

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281 **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-282 grade cuppers in Jimma ECX center. Acidity, body, cup cleanness and flavor were assessed 283 following a standard method (ECX, 2011). This Q-grade standard method involves Q-certified 284 cuppers, i.e., cuppers licensed by the Specialty Coffee Association (SCA) Coffee Quality 285 Institute (CQI). Roasting, grinding, and brew preparation: This was performed by the ECX 286 laboratory in Jimma, Ethiopia. A roaster equipped with a cooling system, in which air was 287 forced through a perforated plate, capable of roasting up to 500 g of coffee beans, was used for 288 roasting the coffee beans. An amount of 100 g green beans was used for each sample and the 289

beans were put into the roasting machine with six cylinders (Probat, 4 Barrel Roaster, 290 Germany). They were carefully roasted for 7-8 minutes to medium roast at temperatures of 291 200°C. Subsequently, the roasted bean samples were ground to a medium level using a 292 Guatemala SB electrical grinder, which were cleaned well after each sample. The medium 293 roasted coffee was tipped out into a cooling tray and allowed to cool down for 4 minutes rapidly 294 by blowing cold air through it. Then, eight grams of coffee powder was put into a 250 mL cup 295 and 5 cups per coffee sample were used. Next 125 ml boiled water (93°C) was poured onto the 296 ground coffee, followed by stirring the content to ensure homogeneity of the mixture. Then, 297 298 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 299 were cup tasted by three professional Q-grade cuppers operating in ECX. Each panelist gave 300 their independent judgment using a cupping form and the average score of the three cuppers 301 was used. 302

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Finally, the total preliminary quality was calculated using raw and cup quality scores. Coffee 304 samples of grades 1-3 (specialty 1, 2 and 3) were assessed for total specialty quality. 305 Accordingly, aroma, flavor, acidity, body, uniformity, cup cleanness, overall preference, 306 307 aftertaste, balance and sweetness were rated on a scale from 0 to 10. The sum of all these cup specialty 100 308 quality attributes gave а quality ranging from 0 to 309 (https://sca.coffee/research/protocols-best-practices).

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311 Statistical analyses

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In order to establish the relationship between shade tree species and soil biogeochemistry, shade tree species and coffee quality attributes, and finally soil biogeochemical and coffee quality attributes, two statistical approaches were chosen to analyze the data.

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First of all, a linear-mixed effect model was used to quantify shade tree species effects on soil biogeochemistry and coffee bean quality attributes. The models were fitted using maximumlikelihood methods in the 'lme4' packages using the 'lmer' function (Harrison *et al.* 2018) and always included the location as random-intercept term. The p-values of the fixed effect (shade tree species) was 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. 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, 2017).

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Secondly, first Principal Component Analyses (PCA) was performed on the soil chemical 330 variables and then the first axes of the PCA then used to reveal relationships between soil 331 332 biogeochemistry and coffee quality attributes. To select prominent variables for subsequent regression analyses, the first two principal components, accounting for 64.7% of the variation, 333 from soil biogeochemistry were taken (Fig. 3) and the scores obtained from the above 334 considered principal components were utilized as independent variables in linear mixed-effect 335 models (LMMs) to test their effects on coffee quality attributes using the backward variable 336 selection procedures using the function 'prcomp' in 'factoextra' package. In this hierarchal 337 nested design, the 28 locations are considered as blocks (random variable) whereas the 338 339 individual coffee plants are considered as replicates.

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341 **3. Results**

342 **3.1.** Linking soil biogeochemistry to shade tree species

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Except for soil C, all the remaining soil chemical variables showed a significant shade tree-344 species effect (p<0.05) (Supplementary table 1). Available and total phosphorus values were 345 highest under *Albizia*, whereas the remaining shade tree species appear to have similar contents 346 of available and total phosphorus (Fig. 2). Canopy cover as a response variable was included 347 in supplementary table 1 and Fig. 2 to show whether shade tree species affect the canopy cover: 348 canopy cover was unaffected by the shade tree species. Based on 13 soil chemical variables 349 studied, shade tree species comparisons revealed that Albizia gummifera and Cordia africana 350 were most often significantly different from Acacia abyssinica and Croton macrostachyus do 351 (Table 1 & Fig. 2). Available-P (Olsen, lactate and oxalate) and total-P were higher under 352 Albizia plots than the other shade trees (Fig. 2). The four shade tree species mostly overlap also 353 in the PCA analysis (Fig. 3), suggesting similarity between them in explaining soil chemical 354 characteristics except soil available P, Ca and Mg. There were no significant effects of the 355 shade tree species on the cupping quality. Only the 100-bean weight was significantly affected 356 by the shade tree species (Fig. 4). 357

Shade tree species	Soil biogeochemical variables												
	%C	%C:N	pН	Lactate	Lactate	Lactate	Lactate	Oxalate	Oxalate	Olsen	Oxalate	Lactate	Total
			(H_2O)	Κ	Mg	Ca	Al	Al	Fe	Р	Р	Р	Р
Acacia abyssinica													
Mean	5.8	12.3	4.7	596.0	176.8	1233.7	758.3	2883.0	5854.0	126.9	713.3	121.6	1724.8
SE	0.2	0.1	0.1	38.7	15.2	122.0	32.6	82.6	127.9	17.9	80.5	18.1	107.2
Albizia gummifera													
Mean	6.1	12.5	4.8	678.6	167.8	1632.1	652.4	2693.8	6600.0	209.7	1147.4	223.3	2304.5
SE	0.2	0.1	0.1	44.2	14.2	223.1	17.4	50.3	175.8	25.8	118.9	28.8	154.3
Cordia africana													
Mean	5.5	12.8	5.6	952.1	343.9	2797.6	496.5	2385.6	5859.7	113.5	804.6	147.7	1682.3
SE	0.2	0.2	0.1	49.7	28.9	231.3	36.3	83.5	242.5	12.1	78.0	14.9	121.9
Croton macrostachyus													
Mean	5.1	12.3	5.7	931.4	361.2	2601.0	617.6	2823.8	5368.8	84.1	649.2	126.1	997.0
SE	0.2	0.2	0.1	44.1	26.0	262.8	39.3	89.6	166.9	15.9	97.4	26.6	88.1

Table 1. Descriptive statistics of the studied shade tree species on soil biogeochemical variables

SE = *standard error*

3.2. Linking coffee bean quality attributes to soil biogeochemistry

A PCA condensed soil chemical variables into axes representing a gradient of soil biogeochemistry, in which the length of the vector indicates the variance explained by the variables (Fig. 3). Values of phosphorus obtained by different extraction methods (Olsen-P, lactate-P, oxalate-P and total-P) are strongly and positively correlated to each other. The 1st axis, explaining 39.4% of the variance, runs from high nutrient content of the plots (i.e. high lactate-Ca and Mg, and pH) to high P-rich plots (negative axis) (Fig. 3). Meanwhile, the soil chemical variables clustered to the right (lactate-Ca, lactate-Mg and pH) have large positive loadings on the first component, i.e., the relationship among these variables is strong and positive. The 2nd axis, explaining 25.3% of the variance, separated relatively N-rich/high OC content (positive axis values) from Al-rich (negative axis values) plots. This axis seems to be mostly driven by Al, which in turn is negatively correlated to all coffee quality attributes (Fig. 5).





Fig 2. Effect of four shade tree species on various soil biogeochemistry and canopy cover. Bars denote standard errors and the different letters denote significant differences among shade tree species.



Fig 3. PCA biplot showing groupings and relationships among soil biogeochemical variables per shade tree species.

A significant effect of the first principal component (PC1) was observed on total preliminary quality (p=0.032, R²m=0.054, R²c=0.42) (Fig. 5). However, the effect size and beta-value are very small. Likewise, specialty quality was found to be significantly affected (p=0.046, R²m=0.017, R²c=0.15) by PC1, whereas hundred bean mass was found to be significantly affected (p=0.043, R²m=0.048, R²c=0.11) by PC1 (Fig. 5). There was also a significant effect of the second principal component (PC2) on total preliminary quality (p=0.012, R²m=0.044, R²c=0.34), specialty quality (p=0.033, R²m=0.086, R²c=0.23) and hundred bean mass (p=0.023, R²m=0.18, R²c=0.29) by PC2 (Fig. 5).



Fig 4. Boxplot representing the effect of four shade tree species in three coffee bean quality attributes. Mean values and standard error bars represent the total preliminary and specialty quality, and value of hundred bean mass (g).

4. Discussion

4.1. Shade tree species have a significant impact on soil biogeochemistry

Our present study demonstrates that the individual shade tree species have a substantial effect on almost all selected soil chemical variables. Particularly, lactate- Ca and pH are higher under *Cordia africana*, implying that plots under these trees appeared to be less acidic as compared to the plots under N-fixing shade tree species. Although shade tree-coffee association has been shown to reduce soil acidity, the selection of specific shade tree species could have differing impact on the changes in pH (Muchane et al. 2020; Rigal, 2018). In this particular study, soils under leguminous shade trees (Acacia and Albizia) were found to be more acidic. Likewise, studies involving shade tree species have also reported a consistent result: that leguminous shade trees have been shown to contribute to a lower soil pH when compared to non-N-fixing tree species (Tully et al. 2012; Pinho et al. 2012). In contrast, a reduced soil acidification was reported under some N-fixing shade trees (Sileshi et al., 2014; Wang et al. 2010). This might be due to the fact that tropical leguminous shade trees could take-up less cations and this might have a reduced acidifying effect on the rhizosphere because the amino acids produced by Nfixation have a lower tendency to release protons (Wang et al. 2010). On the other hand, the release of H⁺ during N-fixation must also be considered (Liu et al. 1989). Besides, trees could minimize soil acidification both by decreasing drainage and through uptake of otherwise leached nutrients. In tropical and sub-tropical forest ecosystems, a paradoxical relationship is commonly observed between shade tree species and soil acidification. For instance, a subtropical legume tree (Senna siamea) has been shown to recycle calcium from deeper soils and significantly reduce acidity of the top soil (Vanlauwe et al. 2005). Hence, the soil acidity alleviating effect of shade tree species depends on the litter chemical composition, together with N fixing ability (Wong et al., 2002).

C:N ratios were also highest under *Cordia africana*. This ratio is an important indicator of the rate of decomposition, particularly residue-cover on the soil and crop nutrient cycling (predominantly N) (Adetunji *et al.* 2020; Ashworth *et al.* 2020). The C:N ratio in this particular study had a negative relationship with Aluminum, implying that Aluminum rich plots can have a negative influence on soil biology which in turn negatively affect the decomposition rates (Rowe *et al.* 2013; Aitkenhead-Peterson *et al.* 2012). Our present findings contradict the findings of Etafa (2022), who reported that soil organic matter, total N, available P, exchangeable K, and soil pH were generally greater under shade trees under study.

We also find that the N-fixing leguminous tree *Albizia* enhanced soil available P compared to the non-N-fixing shade tree species. This effect was also present in Acacia, but to a lesser extent. This agrees with the previous findings of Aleixo et al. (2020), who reported that soil P availability was improved by N-fixing leguminous trees. This positive association between soil P availability and N-fixing leguminous trees could be due to the conditions favoring the production of extracellular phosphatase enzymes by N-rich plant and microorganism species in the rhizosphere and bulk soils. As a result of such environmental conditions, P mineralization could increase, and, thus P-availability in the soils would increase. In general, the association of N and P in agroforestry systems with N-fixing leguminous trees is very strong (Aleixo et al. 2020; Treseder and Vitousek, 2001). However, this depends on different environmental conditions (mainly climate and soil), cropping system and management practices (Aleixo et al. 2020). Although soils differ widely in their P content, generally the range of total-P in many soils is in the range of 200-800 mg/kg (Qihua et al. 2020; Cross and Schlesinger, 1995). However, the quantities of soil total-P in the present study was considerably higher than expected (600-2500 mg/kg soils) under N-fixing shade trees, Acacia and Albizia (Fig. 2). This could be associated with the application of large quantities of chemical inputs to the soils of the plantation coffee in the study plots. Most importantly, too much application of phosphatic and potassium fertilizers (75 g NPK/tree/year), which is equivalent to 250 kg NPK/ha, in our study plots most likely is the consequence for the high values of total-P. In addition to this, the high total-P values obtained in this particular study could be partly explained by the high values of percent soil organic carbon. Soil organic carbon content has an important role in relation to the content of P in soils: a strong positive correlation exists between P content and organic carbon of the soils under study (Kang et al. 2009).



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Fig 5. Relationship between coffee quality attributes and soil chemical characteristics (quantified via PC1 and PC2 from the soil chemical PCA depicted in Fig. 3). Data points represent a particular response variable at a single coffee tree (n=84) in which the fitted regression lines and 95% confidence intervals are from linear mixed-effect models at p<0.05. R^2m = marginal R^2 (is the proportion of variance explained by the PCA score values; R^2c = conditional R^2 (is the proportion of variance explained by the PCA score values).

When correlating the different P-extraction methods with each other, the overall picture indicates that the three extraction methods are strongly correlated (Fig. 3). How these variables occur concurrently influences the solubility of phosphates and hence the extractability and efficiency of different extraction methods are required to estimate plant-available soil P (Fath *et al.* 2019; Penn and Camberato, 2019). The availability of P in soils is influenced by soil pH and the presence of Fe and Al (Penn and Camberato, 2019). This was shown by our present findings in that Al-rich plots and P-rich plots (Olsen-P, lactate-P, oxalate, and total-P) are negatively associated with each other.

4.2. Soil biogeochemistry affect coffee bean quality in a plantation coffee

Our present findings are consistent with that of our own previous work in the same region in the sense that most soil chemical variables were found to have a significant positive association with hundred bean mass (Getachew *et al.* 2022). Similarly, total preliminary and specialty quality had a significant positive relationship with soil chemical variables (Getachew *et al.* 2022).

Besides, recent studies from southwest Ethiopia revealed that soil fertility variables 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). They primarily reported that coffee with improved cup quality was collected from coffee farms with greater available P, K, Mg, and Zn levels. These 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). Castro-Tanzi *et al.* (2012) have pointed out that, excessive NPK fertilizer use and increased aluminum toxicity were linked to a lower coffee cup quality. Another study shows that coffee cup quality was significantly and Positively associated with available P and K (Yadessa *et al.* 2020). Meanwhile, our results clearly indicate that the

conditional R^2 values are far higher than the marginal R^2 values, implying that the spatial random variability is more important than the variability due to the fixed effects (Supplementary table 1 and Fig. 5).

4.3. Shade tree species have limited benefits for coffee bean quality improvement

Our data suggest that shade tree species have a limited effect on coffee bean quality attributes. Except hundred bean mass, no associations were found between coffee bean quality attributes and shade tree species identity (Fig. 4). This contrasts with previously reported findings from the same region but from different coffee agroecosystems that found significant effect of shade tree species on coffee bean quality attributes (Yadessa et al. 2008). If soil characteristic improvements by the shade tree species were to improve coffee bean quality attributes, we would expect to find greater coffee bean quality in locations with better soil characteristics. However, we could not confirm from our present data whether the observed effect of soil biogeochemistry on bean quality attributes are associated with the shade tree species or not. This indicates that, shade tree species are unlikely to be the factor most limiting coffee bean quality attributes in our study area. However, a limited effect of shade tree species identity was observed on hundred bean mass (Fig. 4), which could be due to the increasing competition for light as a shade canopy cover increases (Getachew et al. 2022, Lin, 2010). If shade trees are to be included in coffee agroforestry systems, the tree shade cover need to be taken into consideration, while maintaining other benefits of shade trees like microclimate buffering. Based on the evidence provided by the present findings, it appears easy to justify the implementation of shade trees for soil fertility improvements for a long-term coffee sustainability. Yet, also the interaction between the elevation where the coffee tree is grown and the shade tree canopy cover need to be considered as an important environmental driver (Getachew et al. 2022).

4.4. Implications for the coffee producers in the study area

Farmers' incentives for planting shade trees are diverse other than the cup quality of coffee. Besides the observed effect of shade tree species on physical bean quality, a coffee producer's decision to plant shade trees could also depend on a number of factors, such as certification opportunities (e.g. Rainforest Alliance, etc.), temperature buffering, management considerations related to agronomic inputs, and the need for alternative products from the trees. Hence, there is a need to weigh the effects of shade tree species from multiple perspectives. Our findings show that shade tree species had a significant effect on soil biogeochemistry but only a limited effect on coffee bean quality attributes (except in the bean mass). Although our data did not allow us to identify the mechanisms that caused the observed effects of soil biogeochemistry on coffee bean quality attributes, we found a positive and significant effect of soil nutrient status on total preliminary, specialty quality, and hundred bean mass. The findings of this study show that shade trees can be planted with the goal of improving soil biogeochemistry, and that tree species recommendation for coffee agroecosystem need to be associated to climate buffering, carbon sequestration and pathogen loads while taking into account a wide range of cropping systems and climatic zones. In the future, further research should focus more on coffee-shade tree associations such that our understanding of the biogeochemical impacts can be improved, especially given the microclimatic importance of shade tree species in buffering the negative impacts from heatwaves and droughts due to climate change.

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