

## Research Article

# Grain Yield and Nitrogen Uptake of Maize (*Zea mays* L.) as Affected by Soil Management Practices and Their Interaction on Cambisols and Chernozem

Ashenafi Nigussie <sup>1</sup>, Wassie Haile <sup>2</sup>, Getachew Agegnehu <sup>3</sup>, and Alemayehu Kiflu <sup>2</sup>

<sup>1</sup>Department of Natural Resource Management, Wondo Genet Agriculture Research Center, Shashemene, Ethiopia

<sup>2</sup>School of Plant and Horticultural Sciences, Hawassa University College of Agriculture, Hawassa, Ethiopia

<sup>3</sup>Department of Natural Resource Management, Holeta Agriculture Research Center, Holeta, Ethiopia

Correspondence should be addressed to Ashenafi Nigussie; [nigussie.ashenafi@yahoo.com](mailto:nigussie.ashenafi@yahoo.com)

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Although numerous factors contribute to wide yield gaps, low external inputs, particularly N, and poor cropping practices such as soil tillage and monocropping are among the major factors affecting low maize production. In view of this, field experiments were implemented on two sites with Cambisols and Chernozem soil types in two consecutive years to evaluate the impacts of different soil management practices on the grain yield and quality, nitrogen uptake, and selected soil properties. A three-factor experiment was arranged as a split-split plot arrangement randomized complete block design with three replications. The minimum tillage (MT) and conventional tillage (CT) were used as the main plot, haricot bean-maize rotation and maize monocropping were used as the subplot, and four levels of nitrogen fertilization (control, 20 t ha<sup>-1</sup> compost, 46 kg N ha<sup>-1</sup> + 10 t ha<sup>-1</sup> compost, and 92 kg N ha<sup>-1</sup>) were used as the sub-subplot. Analysis of variance showed that soil management practices were significantly affecting grain yield, N-uptake, and soil properties. In sites, the conventional tillage and rotation system increased the grain yield and N-uptake in contrast to the minimum tillage and monocropping, respectively. Similarly, nitrogen evidently affected the grain yield, N-uptake, and selected soil properties. However, tillage methods differed in their effects on soil chemical properties; soil organic carbon and total nitrogen concentrations were improved through MT compared to CT. Grain yield was significantly associated with NDVI, grain N-content, and N-uptake. Therefore, a CT plus haricot bean-maize rotation system with the addition of solely 92 kg N ha<sup>-1</sup> and integrated 46 kg N ha<sup>-1</sup> + 10 t compost ha<sup>-1</sup> could be recommended for Hawassa Zuria (Cambisols) and Meskan (Chernozem) districts, respectively. However, in order to ensure sustainable maize production in the investigated areas, an integrated N treatment with MT and a rotation system may be recommended, which could improve soil properties.

## 1. Introduction

Maize or corn (*Zea mays* L.) is one of the world's leading cereals, ranking second in production after wheat [1]. Ethiopia is the seventh maize-producing country in Africa. It is the second in area coverage next to teff (*Eragrostis tef* (Zucc.)), with a total land area of 10,478,217 ha being under cereals, of which maize covered about 17.68% (2,274,305.93 ha) [2]. Despite the large area under maize production, its current national average yield is about 4.2 t ha<sup>-1</sup> [2], which is far below the world's average yield of

5.8 t ha<sup>-1</sup> [1]. Although numerous factors contribute to wide yield gaps, low external inputs, particularly N, poor soil fertility, reduced water-holding capacity of the soil, and poor soil infiltration problems are among the major factors paid for low maize productivity [3–5]. Moreover, frequent tillage, monocropping, and complete removal of crop residues are also the governing factors for low productivity [6].

Tilling soil is the most universally used agricultural practice and has been considered as a “Farmer's technology” for at least 10,000 years [7]. Tillage is an important soil management practice for successful crop production. It

provides various benefits to farmers [8], which also negatively affects soil resources and the environment (Gupta et al., 2002). It contributes up to 20% of yield reduction [9] and affects N dynamics in the soil by influencing organic matter (OM) decomposition, soil aeration, compaction, rooting pattern, and microbial activity [10, 11]. Similarly, Ozpinar and Cay [12] and Pekrun et al. [13] proved that adopting different tillage systems has effects on plant nutrient dynamics and the distribution of macronutrients and micronutrients in topsoil. Studies made by other authors also emphasized the impacts of different tillage practices on maize grain yield and its components [11, 14].

Conventional tillage (CT) is a frequently used tillage method, which primarily improves the soil's physical properties [14]. However, CT has the potential to reduce soil organic matter due to enhanced decomposition rate and, hence, negatively affect long-term crop productivity, nutrient uptake, and soil health [15]. The previous study also confirmed that organic matter mineralization is enhanced through conventional tillage [16, 17]. Nowadays, conservation tillage systems such as "minimum" and "zero tillage" have entered widespread use in most farmers around the globe, due to their benefits in minimizing soil erosion, preserving soil moisture, improving soil organic matter, and reducing labor, fuel, and machinery costs [18, 19]. However, at the transitional time, yield, nutrient bioavailability, particularly N, is commonly lower in minimum or zero tillage than the conventional method [20].

Crop rotation is a systematic approach that allows preserving the existing natural resources and their efficient utilization [21]. At present, agricultural researchers have given great emphasis to crop rotation due to its effects on N efficiency and nitrogen availability to the plant [22]. An earlier study proved that the cost of mineral nitrogen fertilizer requirements of grain crops could be reduced by 4 to 71% due to legume-based crop rotation [23]. Correspondingly, Fustec et al. [23] reported that the nutrient status in soil and its availability to succeeding crops are affected by cropping systems. The legume-based rotation systems can improve the yield of succeeding grain crops and have the potential to minimize N-losses compared to monocropping [24]. Berzsenyi et al. [25] reported that the grain yield produced from the rotation system was higher than monocropping under the same condition. Therefore, the presence of legumes in the cropping system is an environmentally beneficial and economically sound approach [23, 26].

The application of nutrients, mainly nitrogen, is the second precondition for effective maize production. Nitrogen (N) is a generally deficient element in all agricultural soils and cropping systems of the world [27]. Therefore, nitrogen management in maize cultivation is critical to increasing productivity and nutritional quality. Previous studies revealed that soil fertility significantly improved due to N management [28]. On the one hand, limited use of inorganic N fertilizers led to yield reduction [29, 30]. On the other hand, the excess application is uneconomical, environmentally unsafe, and potentially harmful to crops [31]. To prevent these problems, the integrated use of organic and

inorganic N sources is a good framework to improve grain yield and N-uptake and reduce N-losses. Also, there is a need to integrate different soil management practices to improve grain yield and N-uptake (Kumar et al., 2015).

In our country, however, there is scarce information about the effects of tillage, cropping systems, nitrogen fertilization, and their interaction on the yield, nitrogen uptake of maize, and soil chemical properties. Therefore, this study was instigated to evaluate the effects of different soil management practices on the maize grain yield and quality, nitrogen uptake, and selected soil chemical properties in the Central Rift Valley of Ethiopia, under two soil types, namely, Cambisols and Chernozem.

## 2. Materials and Methods

**2.1. Description of the Experimental Sites.** The field experiments were conducted for two consecutive years (2019 and 2020) in Hawassa Zuria and Meskan districts of the Central Rift Valley of Ethiopia. The Hawassa Zuria site is geographically situated at 07° 1' 0.83" N latitude and 38° 22' 26" E longitude with an altitude of 1,713 m above sea level (asl). The site is mainly characterized by a semiarid climate with a long-term average annual rainfall of 958 mm, of which 81% falls during the growing season (April to October) and an annual mean temperature of 21°C (Figure 1). The experimental site at Meskan is found at 08° 05' 33" N latitude and 38° 26' 75" E longitude with an altitude of 1,841 m asl. The experimental site is mostly categorized under a semiarid climate with a long-term average annual rainfall of 987 mm, of which 84% falls during the growing season (April to October) and an annual mean temperature of 20.4°C (Figure 1). The soil types for the field trial were Cambisols for Hawassa Zuria and Chernozem for Meskan, according to the WRB soil classification system (IUSS Working Group, 2015).

The study sites were selected purposively on the basis of their potential for maize production and their difference in soil fertility status. The major crops grown in the study areas include maize (*Zea mays* L.), sorghum (*Sorghum bicolor*), haricot bean (*Phaseolus vulgaris*), and millet (*Eleusine coracana*). Farmers in the study areas usually used blanket recommendations of urea and NPS inorganic fertilizers as sources of nitrogen and phosphorus, respectively. In both sites, maize was the preceding crop with conventional tillage and monocropping practices.

**2.2. Physicochemical Properties of Experimental Soils and Compost.** Prior to setting the treatments, representative 12 random soil samples were collected from 0 to 20 cm soil depth in March 2019 to measure the baseline values at each experimental site, following the standard soil sampling procedure. After physical homogenization, representative three composite subsamples per site were prepared for physicochemical analysis. The samples were pulverized and sieved through a 2 mm sieve after being air-dried at room temperature. However, 0.5 mm mesh wire was used for the determination of organic carbon (OC) and total nitrogen (TN). The soil laboratory analysis was executed in the

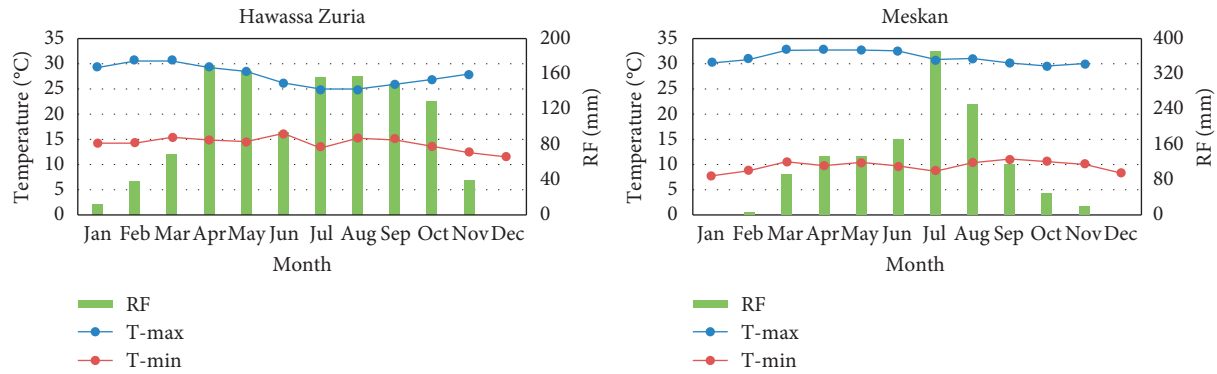


FIGURE 1: Monthly average temperature and annual rainfall of the study areas (2019 and 2020). RF: rainfall; T-min and T-max: minimum and maximum temperatures, respectively.

Laboratory of Hawassa University College of Agriculture. Selected soil physicochemical characteristics at the start of the experiment are shown in Table 1.

The compost was prepared at the Wondo Genet Agricultural Research Center Botanical Garden using locally available composting materials such as green leaves, farmyard manure, animal feed leftovers from dairy cattle, fresh and dry cow dung, bedding materials, and wood ash. Three representative subsamples were used to examine pH value, electrical conductivity (EC), OC, TN, C:N ratio, and available phosphorus (Avail-P). Table 1 also lists the chemical parameters of the compost that were used in this investigation.

**2.3. Treatments and Experimental Design.** Two tillage methods (TM) were evaluated: conventional tillage (CT) and minimum tillage (MT). The two tillage practices were combined with two cropping systems (CS): haricot bean-maize rotation system (RCS) and maize monocropping system (MCS). In addition, four levels of nitrogen fertilization (NF) (0, 20 t compost ha<sup>-1</sup>, 46 kg N ha<sup>-1</sup> + 10 t compost ha<sup>-1</sup>, and 92 kg N ha<sup>-1</sup>) were combined with tillage practices and cropping systems (Table 2). Treatments were arranged as split-split plot arrangement randomized as a randomized complete block design (RCBD), with tillage methods as the main (whole) plots, cropping systems as subplots, and nitrogen fertilization treatments as sub-subplots, with three replicates, making 48 sub-subplots for each experimental site.

**2.4. Experimental Procedures and Management Practices.** Tillage methods as the main plots and cropping systems as subplots were arranged in a RCBD with three replications during the 2019 cropping season. The experimental plots assigned for conventional tillage were plowed three times before seed sowing using an ox-drawn local Maresha, following optimum sowing time. Plots intended for minimum tillage, on the other hand, were plowed once during seeding with an ox-drawn local Maresha. Moreover, minimum tillage plots received one application of Roundup herbicide

(glyphosate) (3 liters per hectare) to control weeds before seed emergence.

A recently released hybrid maize variety “BH 546” and haricot bean variety “Hawassa Dume” are well adapted to the prevailing agroecological conditions and were sown at optimal sowing time. Maize and haricot beans were sown at a space of 80 cm × 25 cm and 40 cm × 10 cm, respectively. Each main plot and subplot had an area of 15 m × 9 m = 135 m<sup>2</sup> and 15 m × 4 m = 60 m<sup>2</sup>, respectively. The total experimental area was 31.5 m × 30 m = 945 m<sup>2</sup>.

Phosphorus fertilizer was applied to all plots during seed sowing as triple superphosphate (TSP) at the recommended rate (46 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), in a band in the row. To minimize N-losses and increase their efficiency, urea fertilizer was applied at the rate of 92 kg N ha<sup>-1</sup> in the split form: half at sowing time and the remaining half at the vegetative growth stages of six leaves (V6) of the maize, in all plots except the sole bean, which is in bean-maize rotation treatment, assuming the bean benefited from its N-fixation. As required, recommended agrotechnical measures were performed evenly in all experimental units. Furthermore, 30% of the crop residues were retained after harvesting in minimum tilled plots.

During the 2020 cropping season, the experiment was laid out in a 2 × 2 × 4 split-split plot arrangement in a RCBD, with three replications. Each main plot (conventional and minimum tillage methods) had eight treatment combinations, i.e., two cropping systems with four nitrogen fertilization treatments. According to the treatment, ten days before sowing, compost was applied on the surface of the soil, based on inorganic N equivalency. In the case of conventionally tilled plots, applied compost was incorporated (0–20 cm depth) following the application on the top of the soil using ox-drawn local Maresha. At the minimum tilled plots, the compost was evenly distributed on the surface of the soil and the incorporation was made during sowing since in the minimum tillage method we proposed to till the soil once that is during seed sowing using ox-drawn local Maresha.

The hybrid maize variety BH 546 was used as the test crop. Similarly, the hybrid maize variety BH 546 was used as the test crop. The pathways between blocks and plots were 1.5 m and 1 m, respectively. Each sub-subplot had a size of 4.8 m × 3 m (14.4 m<sup>2</sup>) and accommodated six maize rows

TABLE 1: Some physicochemical properties of the surface layer of soils (0–20 cm) and compost prior to treatment application.

Soil properties	Units	Site/soil type		
		Hawassa Zuria Cambisols	Meskan Chernozem	Compost
Physical properties				
Sand	%	42	16	—
Silt	%	32	36	—
Clay	%	26	48	—
Textural class	—	Loam	Clay	—
Chemical properties				
pH	—	5.86	6.57	6.79
EC	ds/m	0.03	0.06	0.09
OC	%	2.42	4.10	14.80
TN	%	0.26	0.37	1.21
C:N	—	9.31	11.10	12.23
Avail-P	mg kg <sup>-1</sup>	4.52	23.74	77.10
CEC	cmol <sub>c</sub> kg <sup>-1</sup>	20.00	62.00	—
Exchangeable Ca	cmol <sub>c</sub> kg <sup>-1</sup>	8.27	39.33	—
Exchangeable Mg	cmol <sub>c</sub> kg <sup>-1</sup>	0.34	7.67	—
Exchangeable K	cmol <sub>c</sub> kg <sup>-1</sup>	2.03	2.20	—
Exchangeable Na	cmol <sub>c</sub> kg <sup>-1</sup>	1.85	1.01	—

TABLE 2: Arrangement of experimental treatments.

Treatment no.	Tillage methods (TM)	Cropping systems (CS)	Nitrogen fertilization (NF)	Treatment combinations
1	MT	RCS	N1	MT + RCS + N1
2	MT	RCS	N2	MT + RCS + N2
3	MT	RCS	N3	MT + RCS + N3
4	MT	RCS	N4	MT + RCS + N4
5	MT	MCS	N1	MT + MCS + N1
6	MT	MCS	N2	MT + MCS + N2
7	MT	MCS	N3	MT + MCS + N3
8	MT	MCS	N4	MT + MCS + N4
9	CT	RCS	N1	CT + RCS + N1
10	CT	RCS	N2	CT + RCS + N2
11	CT	RCS	N3	CT + RCS + N3
12	CT	RCS	N4	CT + RCS + N4
13	CT	MCS	N1	CT + MCS + N1
14	CT	MCS	N2	CT + MCS + N2
15	CT	MCS	N3	CT + MCS + N3
16	CT	MCS	N4	CT + MCS + N4

with inter- and intrarow spacing of 80 and 25 cm, respectively. Each row and plot had 12 and 72 plants, respectively. Phosphorus fertilizer was applied during seed sowing to all plots as triple superphosphate (TSP) at the recommended rate (46 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). Nitrogen fertilizer (urea) was applied in the split form: half at sowing and the other half at the vegetative growth stages of six leaves (V6) of the maize according to the treatments. Other agronomic practices were carried out uniformly in all experimental units.

After experimentation, soil samples were collected from each experimental unit and location, and then, the collected samples were prepared for selected chemical analysis.

**2.5. NDVI and Grain Yield Measurements.** The normalized difference vegetation index (NDVI) was measured from the central two rows at the vegetative growth stages of six (V6)

and eight leaves (V8) using a handheld Green Seeker<sup>TM</sup> optical sensor unit (NTech Industries, Inc., USA) following the method used by Verhulst et al. [32], and their mean was taken for computation. At the Hawassa Zuria and Meskan trial locations, samples of maize grains were gathered at physiological maturity, which corresponded to 173 and 175 days after sowing, respectively. The samples were collected from a net plot area of 4 m<sup>2</sup> (1.25 m × 3.2 m) by rejecting the border rows, from three replications. The harvested grain yield was adjusted to a 12.5% moisture level [33], and it was converted into hectare bases. Twenty grams of grain samples was taken from each experimental unit. The grains were oven-dried to constant weight thereafter, and the samples were ground and passed through a 0.5 mm sieve. The nitrogen content in the grain was analyzed using the Kjeldahl procedure after wet digestion by H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> [34].

TABLE 3: Analysis of variance of NDVI, grain yield, nitrogen uptake, and protein content of maize grown at the two sites.

Source variation	DF	Hawassa Zuria					Meskan				
		NDVI	GY	GNC	GNU	GPC	NDVI	GY	GNC	GNU	GPC
TM	1	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
CS	1	**	**	**	**	**	ns	ns	**	**	**
NF	3	***	***	***	***	***	***	***	***	***	***
TM×CS	1	ns	ns	*	*	*	ns	ns	ns	ns	ns
TM×NF	3	ns	ns	*	*	**	ns	ns	ns	ns	ns
CS×NF	3	**	ns	ns	ns	ns	ns	ns	**	*	**
TM×CS×NF	3	*	ns	*	*	*	ns	ns	ns	ns	ns
Replication	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep.×TM	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep.×TM×CS	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)		4.1	4.6	6.9	8.3	6.8	2.9	5.2	3.8	5.6	3.8

DF: degree of freedom; NDVI: normalized difference vegetation index; GY: grain yield; GNC: grain N-content; GNU: grain N-uptake; GPC: grain protein content; CV: coefficient of variation. \*Significant at  $P \leq 0.05$ ; \*\*significant at  $P \leq 0.01$ ; \*\*\*significant at  $P \leq 0.001$ ; ns: not significant.

**2.6. Nitrogen Uptake and Grain Protein Content.** The grain nitrogen uptake was calculated by multiplying N contents ( $\text{g kg}^{-1}$ ) in grains with the respective grain yield ( $\text{kg ha}^{-1}$ ):

$$\text{grain N uptake} \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{(\text{grain N contentg/kg} \times \text{grain yieldkg/ha})}{1000}, \quad (1)$$

$$\text{grain protien content (\%)} = \text{grain N content (\%)} \times 6.25.$$

**2.7. Data Analysis.** Before the analysis of variance (ANOVA), the normality of the data was checked using the Shapiro–Wilk normality test. Despite the field experiments were carried out for two consecutive years, only the last year's data were used for statistical computation since the third factor (NF) applied in the second season (during 2020). Moreover, the two experimental sites were distinctly different in their soil fertility status (Table 1); subsequently, the statistical analysis was performed independently for each location, using the SAS 9.3 software package [35], considering the experimental treatment as a fixed factor and replication as a random factor. At a probability level of  $P \leq 0.05$ , differences between treatment means were separated using the protected Fisher's least significant difference (LSD) [36]. The LSDs for the main factors and interaction effect comparisons were calculated using the appropriate standard error terms. Pearson's correlation coefficients ( $r$ ) were performed using SAS software 9.3 [35].

### 3. Results and Discussion

**3.1. The Initial Characteristics of the Experimental Soil Types.** The textural class of the soil at Hawassa Zuria was loam, whereas at Meskan clay separately dominates the soil particles and is thus classified as clay (Table 1). The soil pH in  $\text{H}_2\text{O}$  was around 5.86 and 6.57 for Hawassa Zuria and Meskan sites, respectively, and rated as moderately acidic and neutral [37]. The total nitrogen (TN) was higher at Meskan (0.37%) than Hawassa Zuria (0.26%). Similarly, the available P level was lower ( $4.52 \text{ mg kg}^{-1}$ ) at Hawassa Zuria

compared to Meskan ( $23.7 \text{ mg kg}^{-1}$ ). This implies that, in Hawassa Zuria, the soil (Cambisols) is more responsive to nitrogen- and phosphorus-containing fertilizer application than at Meskan (Chernozem soil type). The cation exchange capacity was medium at Hawassa Zuria  $20 \text{ cmol}_c \text{ kg}^{-1}$ , which was attributed to low exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$ , while at Meskan the CEC was  $62 \text{ cmol}_c \text{ kg}^{-1}$  and rated as higher [38]. The initial soil information showed a significant soil fertility variation between the experimental sites, and therefore, it is justifiable to conduct more detailed nitrogen content studies along with different soil management practices.

**3.2. The Effects of Experimental Factors and Their Interactions on Analyzed Parameters.** At Hawassa Zuria, the main effects of TM, CS, and N-fertilization (NF) had a significant effect on maize grain yield (GY), but the interaction of TM×CS, TM×NF, CS×NF, and TM×CS×NF was nonsignificant (Table 3). However, in Meskan, only NF has revealed a significant ( $P < 0.001$ ) effect on the yield, while other main factors and their interactions were nonsignificant (Table 3). The main effects of CS and NF and the interaction of TM×CS, CS×NF, and TM×CS×NF had shown significant ( $P < 0.05$ ) effects on grain N-content (GNC), N-uptake (GNU), and protein content (GPC) in Hawassa Zuria, whereas, in Meskan, grain N-content, N-uptake, and protein content were significantly influenced by the main effects of CS and NF and the interaction of CS×NF (Table 3). At Hawassa Zuria, the main factors of CS and NF and the

TABLE 4: The effects of tillage methods, cropping systems, and N-fertilization on NDVI, grain yield, N-content, N-uptake, and grain protein content of maize at the two sites

Investigated factors	Hawassa Zuria					Meskan				
	NDVI	GY kg ha <sup>-1</sup>	GNC g kg <sup>-1</sup>	GNU kg ha <sup>-1</sup>	GPC %	NDVI	GY kg ha <sup>-1</sup>	GNC g kg <sup>-1</sup>	GNU kg ha <sup>-1</sup>	GPC %
Tillage methods										
MT	0.74	3662.6 <sup>b</sup>	9.6	35.6 <sup>b</sup>	6.0	0.77	7064.0	12.4	89.0	7.8
CT	0.75	3855.5 <sup>a</sup>	9.9	39.1 <sup>a</sup>	6.2	0.78	7094.9	12.5	89.7	7.8
LSD (0.05)	ns	142.6	ns	1.89	ns	ns	ns	ns	ns	ns
Cropping systems										
RCS	0.76 <sup>a</sup>	3835.1 <sup>a</sup>	10.6 <sup>a</sup>	40.9 <sup>a</sup>	6.6 <sup>a</sup>	0.78	7101.0	13.2 <sup>a</sup>	94.5 <sup>a</sup>	8.3 <sup>a</sup>
MCS	0.74 <sup>b</sup>	3683.5 <sup>b</sup>	9.0 <sup>b</sup>	33.7 <sup>b</sup>	5.6 <sup>b</sup>	0.77	7083.2	12 <sup>b</sup>	84.3 <sup>b</sup>	7.3 <sup>b</sup>
LSD (0.05)	0.02	128.8	0.4	3.8	0.24	ns	ns	0.03	5.6	0.2
Nitrogen fertilization										
Control	0.72 <sup>b</sup>	3273.3 <sup>d</sup>	8.2 <sup>d</sup>	26.8 <sup>c</sup>	5.1 <sup>d</sup>	0.74 <sup>d</sup>	5987.9 <sup>d</sup>	11.2 <sup>d</sup>	66.9 <sup>d</sup>	6.9 <sup>d</sup>
20 t ha <sup>-1</sup> compost	0.72 <sup>b</sup>	3628.2 <sup>c</sup>	9.4 <sup>c</sup>	34.2 <sup>b</sup>	5.9 <sup>c</sup>	0.76 <sup>c</sup>	6469.2 <sup>c</sup>	11.8 <sup>c</sup>	75.8 <sup>c</sup>	7.4 <sup>c</sup>
46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	0.77 <sup>a</sup>	3954.4 <sup>b</sup>	11.1 <sup>a</sup>	44.1 <sup>a</sup>	6.9 <sup>a</sup>	0.82 <sup>a</sup>	8169.4 <sup>a</sup>	13.8 <sup>a</sup>	112.5 <sup>a</sup>	8.6 <sup>a</sup>
92 kg N ha <sup>-1</sup>	0.78 <sup>a</sup>	4180.5 <sup>a</sup>	10.5 <sup>b</sup>	44.1 <sup>a</sup>	6.6 <sup>b</sup>	0.79 <sup>b</sup>	7741.8 <sup>b</sup>	13 <sup>b</sup>	102.1 <sup>b</sup>	8.3 <sup>b</sup>
LSD (0.05)	0.03	113.7	0.6	2.62	0.35	0.02	310.4	0.04	4.2	0.25

Values of a parameter means followed by the same letter did not differ significantly across the tillage methods, cropping systems, and N-fertilization at  $P \leq 0.05$  according to the LSD test.

interaction of CS×NF and TM×CS×NF had a significant effect on mean NDVI (Table 3). Likewise, the mean NDVI of the Meskan site was significantly affected by NF and the interaction TM×CS×NF (Table 3).

**3.3. The Influence of Tillage Methods on NDVI, Grain Yield, Grain N-Content and N-Uptake, and Grain Protein Content.** At Hawassa Zuria, tillage had revealed a statistically significant ( $P < 0.05$ ) effect on maize grain yield but not at Meskan despite the higher yield, which was gained from the CT (3855.5 kg ha<sup>-1</sup>) and (7094.9 kg ha<sup>-1</sup>) for Hawassa Zuria and Meskan, respectively (Table 4). In this study, grain yield increased by 5.2 and 0.1% in CT over MT at Hawassa Zuria and Meskan. The positive result of CT on maize grain yield was possibly due to improved soil physical conditions, root growth, infiltration of water, nutrient mineralization, and suppressing weed growth. Our findings were also consistent with other studies conducted on maize in the Central Rift Valley of Ethiopia [39] and teff in the central highlands of Ethiopia [40] and the Tigray of Ethiopia [28]. Correspondingly, Simić et al. [39], Salem et al. [41], and Wang et al. [14] reported that CT in a short-term study increased corn grain yield compared to a minimum or zero tillage due to less soil compaction, which improved soil aeration and organic matter mineralization.

The analysis of variance showed that tillage had no remarkable influence on mean NDVI, grain N-content, and protein content at both sites, although the higher value was recorded in conventional tillage compared to minimum tillage. Similarly, Péter et al. [42] reported no significant observations in their study on the influences of soil tillage and fertilization on the NDVI values of the maize plant. This result is at par with the findings of Habbib et al. [43] who indicated no significant effect of tillage on grain N-content. However, at Hawassa Zuria, tillage had revealed a significant

effect on grain N-uptake, where the higher value was achieved by CT in contrast to MT. Although there were no significant variations observed in grain N-content and N-uptake at Meskan, CT, in general, offered higher values compared to MT (Table 4).

In both locations, the N-content and N-uptake parameters responded positively to CT, possibly due to the stimulation of N-mineralization from organic matter and thereby improved soil mineral N-availability for crop uptake. Similarly, Masvaya et al. (2017) reported that crop yields and N-uptake were superior in CT as compared to minimum tillage. Tilling soils through the conventional method usually improves soil aeration and organic matter decomposition [44]. Similarly, Simić et al. [39] verified the benefit of conventional tillage for better maize grain yield and enhancement in grain protein content. Conversely, minimum soil disturbance resulted in reduced available soil N, which is largely due to an increase in N-immobilization. A similar finding was investigated by Malhi et al. [45] who described that shifting CT to MT tends to decrease nutrient concentrations in the soils and thereby uptake, particularly N, which could be improved through the addition of optimal N and inclusion of legume crops as a precursor.

**3.4. The Effect of Cropping Systems on NDVI, Grain Yield, Grain N-Content, N-Uptake, and Grain Protein Content.** At Hawassa Zuria, the cropping system had considerable ( $P < 0.05$ ) impacts on NDVI, grain yield, grain N-content and N-uptake, and protein content, but at Meskan, except NDVI and grain yield, other parameters were statistically significant (Table 4). The haricot bean-maize rotation system increased maize grain yield, NDVI, N-content, N-uptake, and protein content by 4.1, 2.7, 17.8, 21.4, and 17.9% in Hawassa Zuria and 1.3, 0.25, 10, 12.1, and 13.7% in Meskan, respectively, compared to maize monocropping (Table 4).

This was possibly due to the change in inorganic N-availability in the soil solution caused by previous atmospheric  $N_2$  fixation and legume residue decomposition since legume residues had better quality and a narrow C:N ratio, which results in rapid release of N from the residues [46].

Our result is in covenant with Lafond et al. [47] who stated that legumes offer a positive contribution to soil TN and thus improve its availability. Similarly, Adesoji et al. [48] found improved N-content and N-uptake in maize following soybean rotation system due to enhanced soil N. Correspondingly, Tolera et al. [49] and Yusuf et al. [50] found that the inclusion of pulse crops as a precursor increased corn grain yield compared to maize monocropping.

*3.5. The Effect of Nitrogen Fertilization on NDVI, Grain Yield, N-Content, N-Uptake, and Grain Protein Content.* In our study, the mean NDVI, grain yield, N-content, N-uptake, and grain protein content were higher in the Meskan than in Hawassa Zuria at all treatments of nitrogen fertilization (Table 4). This could be due to the higher initial soil TN and fertility status found at Meskan in contrast to the Hawassa Zuria (Table 1). Analysis of variance depicted that the grain yield differed significantly ( $P < 0.001$ ) among N-treatments in both sites. The grain yield achieved at the control treatment was significantly lower ( $P < 0.001$ ) than the yield realized from either the separate or combined compost or inorganic N fertilizer application. However, the crop response to the applied N-treatments was significantly different between the two sites (Table 4). The highest grain yields of  $4180.5 \text{ kg ha}^{-1}$  and  $8169.4 \text{ kg ha}^{-1}$  were obtained from the application of  $92 \text{ kg N ha}^{-1}$  and  $46 \text{ kg N ha}^{-1} + 10 \text{ t ha}^{-1}$  compost at Hawassa Zuria and Meskan sites, respectively, suggesting that the initial soil TN and fertility status of the studied locations influenced crop response to applied N-treatments. Similarly, Gehl et al. [51] testified that the optimal N rate could be affected by various factors including soil type, tillage, irrigation, fertilizer timing and method, and their interactions.

In our study, maize grain yield significantly improved with improved N-fertilization, which suggests N was a crucial factor for grain yield formation. Similarly, Kaplan et al. [52] proved that the grain yield increased with increasing the N level. The integrated application of compost with inorganic N fertilizer remarkably improved maize grain yield, as presented in Table 4. When compared to the control (nonfertilized plot), the integrated use of  $46 \text{ kg N ha}^{-1} + 10 \text{ t ha}^{-1}$  compost increased grain yield by 20.8 and 36.4% at Hawassa Zuria and Meskan sites, respectively. This could be due to the direct addition of N through the decomposition of the compost added to the soil and soil physical improvement caused by organic input. Similarly, Zahir et al. [53] stated that the combined application of urea and compost at 75:25 or 50:50 ratios (on the N basis) gave superior maize grain yield over that of either a single application or control and thus recommended for yield profitability and sustainable soil productivity.

The addition of  $92 \text{ kg N ha}^{-1}$  and  $46 \text{ kg N ha}^{-1} + 10 \text{ t ha}^{-1}$  compost gave the highest mean NDVI at Hawassa Zuria and

Meskan sites, respectively. The lowest NDVI was recorded in the unfertilized plot (control treatment), followed by sole compost at both locations. The values of NDVI became superior while the nitrogen input increased, indicating that the value improved most likely due to nitrogen availability and uptake. Hailu and Tolera [54] and Baral and Abhikari [55] reported similar findings and indicated that spectral vegetation indices improved with N level, which is useful for acquiring information such as photosynthetic efficiency and potential yield in an indirect way.

Like grain yield and NDVI, N-fertilization had revealed significant effects on GNC, GNU, and GPC (Table 4). In both locations, the integrated use of inorganic nitrogen and compost at a rate of  $46 \text{ kg N ha}^{-1} + 10 \text{ t ha}^{-1}$  remarkably increased GNC, GNU, and GPC by 35.4, 64.6, and 35.3% at Hawassa Zuria and 23.2, 68.2, and 24.6% at Meskan, respectively, when compared to the unfertilized treatment. Our result is in covenant with the findings of Dunjana et al. [56], Negassa et al. [57], and Rusinamhodzi et al. [58], who stated that integrated application of organic and mineral fertilizers at appropriate rates can be an effective approach to improve maize N-uptake. At Hawassa Zuria, the highest grain yield was recorded from the sole inorganic N ( $92 \text{ kg N ha}^{-1}$ ), which is the maximum rate, but lower GNC and GPC were achieved compared to the integrated N-treatment, indicating that the application of  $92 \text{ kg N ha}^{-1}$  was more directed toward grain yield increase than protein content increase in maize grain.

*3.6. The Effects of Interaction of Tillage, Cropping Systems, and Nitrogen Fertilization on NDVI, Grain N-Content, N-Uptake, and Grain Protein Content.* At Hawassa Zuria, the interaction of soil tillage methods and cropping systems with nitrogen fertilization very significantly influenced the mean NDVI, grain N-content, N-uptake, and protein content (Tables 3 and 5). The highest NDVI and grain N-uptake were achieved from the interaction of CT with haricot bean-maize rotation system (RCS) and sole inorganic N  $92 \text{ kg ha}^{-1}$ . At Meskan, however, the three-way interaction of TM, CS, and NF had brought significant ( $P < 0.05$ ) variation on the mean NDVI, as presented in Table 5.

*3.7. Effects of Tillage, Cropping Systems, and Nitrogen Fertilization on Selected Soil Chemical Properties.* At Hawassa Zuria, tillage methods had a considerable effect on soil pH in water but not in Meskan. The higher and lower were for the conventional and minimum tillage, respectively. The previous author also reported similar changes in pH depending on tillage systems [59]. Cropping systems had no significant effect on soil pH at both locations (Table 6). In contrast, the addition of various nitrogen fertilizers showed statistically notable differences in soil pH in both sites. The highest value was observed from sole compost  $20 \text{ t ha}^{-1}$ . The application of compost at the rate of  $20 \text{ t ha}^{-1}$  improved the soil pH by 5% and 6.1% compared to the control treatment at Hawassa Zuria and Meskan, respectively, suggesting that basic cations probably added to the soil solution through the decomposition of compost. This result is in agreement with Ashenafi

TABLE 5: Interaction effects of soil tillage, cropping systems, and nitrogen fertilization on NDVI, grain N-content, N-uptake, and protein content of maize grain.

Investigated factors		Hawassa Zuria				Meskan	
		NDVI	GNC (%)	GNU (kg ha <sup>-1</sup> )	GPC (%)	NDVI	
MT	RCS	Control	0.74 <sup>b</sup>	0.97 <sup>bc</sup>	31.20 <sup>bc</sup>	6.07 <sup>c</sup>	0.74 <sup>c</sup>
		20 t ha <sup>-1</sup> compost	0.71 <sup>b</sup>	1.05 <sup>b</sup>	38.74 <sup>b</sup>	6.60 <sup>bc</sup>	0.78 <sup>bc</sup>
		46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	0.74 <sup>b</sup>	1.23 <sup>a</sup>	48.26 <sup>a</sup>	7.69 <sup>a</sup>	0.84 <sup>a</sup>
		92 kg N ha <sup>-1</sup>	0.74 <sup>b</sup>	1.16 <sup>ab</sup>	47.24 <sup>a</sup>	7.24 <sup>ab</sup>	0.78 <sup>bc</sup>
	MCS	Control	0.71 <sup>b</sup>	0.74 <sup>cd</sup>	23.66 <sup>d</sup>	4.59 <sup>cd</sup>	0.72 <sup>d</sup>
		20 t ha <sup>-1</sup> compost	0.75 <sup>b</sup>	0.79 <sup>cd</sup>	27.43 <sup>c</sup>	4.96 <sup>cd</sup>	0.78 <sup>bc</sup>
		46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	0.78 <sup>a</sup>	0.91 <sup>c</sup>	34.35 <sup>b</sup>	5.67 <sup>c</sup>	0.81 <sup>ab</sup>
		92 kg N ha <sup>-1</sup>	0.79 <sup>a</sup>	0.85 <sup>cd</sup>	33.53 <sup>b</sup>	5.33 <sup>cd</sup>	0.73 <sup>cd</sup>
CT	RCS	Control	0.72 <sup>b</sup>	0.89 <sup>c</sup>	29.93 <sup>bc</sup>	5.58 <sup>c</sup>	0.74 <sup>c</sup>
		20 t ha <sup>-1</sup> compost	0.65 <sup>c</sup>	0.95 <sup>bc</sup>	35.01 <sup>b</sup>	5.90 <sup>c</sup>	0.80 <sup>ab</sup>
		46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	0.80 <sup>a</sup>	1.13 <sup>ab</sup>	46.73 <sup>a</sup>	7.05 <sup>ab</sup>	0.80 <sup>ab</sup>
		92 kg N ha <sup>-1</sup>	0.80 <sup>a</sup>	1.09 <sup>b</sup>	49.86 <sup>a</sup>	6.80 <sup>b</sup>	0.75 <sup>c</sup>
	MCS	Control	0.71 <sup>b</sup>	0.68 <sup>d</sup>	22.32 <sup>d</sup>	4.23 <sup>d</sup>	0.74 <sup>c</sup>
		20 t ha <sup>-1</sup> compost	0.78 <sup>ab</sup>	0.97 <sup>bc</sup>	35.65 <sup>b</sup>	6.06 <sup>c</sup>	0.78 <sup>bc</sup>
		46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	0.77 <sup>ab</sup>	1.18 <sup>ab</sup>	47.13 <sup>a</sup>	7.39 <sup>a</sup>	0.82 <sup>a</sup>
		92 kg N ha <sup>-1</sup>	0.78 <sup>ab</sup>	1.11 <sup>ab</sup>	45.80 <sup>a</sup>	6.94 <sup>ab</sup>	0.78 <sup>bc</sup>

Values of a parameter means followed by the same letter did not differ significantly across the tillage methods, cropping systems, and N-fertilization at  $P \leq 0.05$  according to the LSD test.

TABLE 6: Main effects of tillage, cropping systems, and nitrogen fertilization on soil reaction, organic carbon, total nitrogen, and C : N ratio of the surface layer of soils (0–20 cm).

Treatments	Hawassa Zuria (Cambisols)				Meskan (Chernozem)			
	pH	OC (%)	TN (%)	C : N	pH	OC (%)	TN (%)	C : N
Tillage methods								
MT	6.1 <sup>b</sup>	2.61	0.25 <sup>a</sup>	10.52 <sup>b</sup>	6.8	4.00	0.36 <sup>a</sup>	11.31 <sup>b</sup>
CT	6.2 <sup>a</sup>	2.59	0.23 <sup>b</sup>	11.18 <sup>a</sup>	6.8	3.98	0.33 <sup>b</sup>	12.14 <sup>a</sup>
LSD (0.05)	0.06	ns	0.01	0.33	ns	ns	0.02	0.83
Cropping systems								
RCS	6.1	2.61	0.25	10.72	6.8	3.99	0.35 <sup>a</sup>	11.48 <sup>b</sup>
MCS	6.2	2.60	0.24	10.97	6.8	3.99	0.34 <sup>b</sup>	11.97 <sup>a</sup>
LSD (0.05)	ns	ns	ns	ns	ns	ns	0.01	0.37
Nitrogen fertilization								
Control	6.0 <sup>b</sup>	2.51 <sup>c</sup>	0.21 <sup>c</sup>	11.84 <sup>a</sup>	6.6 <sup>d</sup>	3.83 <sup>d</sup>	0.32 <sup>c</sup>	11.94 <sup>b</sup>
20 t ha <sup>-1</sup> compost	6.3 <sup>a</sup>	2.68 <sup>a</sup>	0.24 <sup>b</sup>	10.73 <sup>b</sup>	7.0 <sup>a</sup>	4.11 <sup>b</sup>	0.32 <sup>c</sup>	13.02 <sup>a</sup>
46 kg N ha <sup>-1</sup> +10 t ha <sup>-1</sup> compost	6.2 <sup>a</sup>	2.66 <sup>a</sup>	0.26 <sup>a</sup>	10.19 <sup>c</sup>	6.9 <sup>b</sup>	4.14 <sup>a</sup>	0.39 <sup>a</sup>	10.57 <sup>d</sup>
92 kg N ha <sup>-1</sup>	6.1 <sup>b</sup>	2.58 <sup>b</sup>	0.25 <sup>ab</sup>	10.63 <sup>bc</sup>	6.7 <sup>c</sup>	3.88 <sup>c</sup>	0.34 <sup>b</sup>	11.37 <sup>c</sup>
LSD (0.05)	0.1	0.04	0.01	0.47	0.08	0.03	0.01	0.53

Within the columns, means followed by the same letters are not significantly different at  $P < 0.05$  according to the LSD test.

et al. [60] and Dikinya and Mufwanzala [61], who reported that organic manure application to the soil tends to increase soil pH due to their microbial decomposition and mineralization and hydroxyl ions released during the mineralization process.

There were no significant changes in organic carbon concentrations across tillage methods and cropping systems in either location (Table 6). This could be due to the fact that the samples were gathered two years after the field trial, which is a short time to oversee the effect of tillage on soil OC. A similar observation was reported by Geisseler and Horwath [62]. If the experimental period extended, the differences in soil OC would be more apparent. However, in both sites MT provided numerically higher OC contents compared to CT. This was theoretically due to the physical

protection of soil OM, residue retention, and reduced soil aeration [63, 64]. Conversely, organic carbon content was significantly affected by N-fertilization (Table 6). The addition of 20 t ha<sup>-1</sup> compost provided the higher OC at Hawassa Zuria, which was statistically comparable with the integrated N-treatment. When compared to the unfertilized (control) plot, compost application increased OC by 6.8%. However, at Meskan, the combined use of compost and inorganic nitrogen fertilizer resulted in the highest level of OC (4.11%), increasing by 8.1% over the unfertilized plot. The present investigation has shown that OC enhanced significantly with the addition of compost. The increase in soil OC after the application of compost is due to the composting material and the rich microbial community, which contributes to the formation of soil organic carbon



[65]. This observation is consistent with the findings of Dhillon et al. [66] and Lorenz and Lal [67], who reported that soil OC content has been enhanced with the addition of either sole or mixed organic inputs.

Tillage practices had a significant effect on soil total N in both locations, with minimum tillage contributing more to total N than the conventional tillage (Table 6). This could be due to enhanced N protection inside microaggregates and macroaggregates, resulting in lower N-losses due to leaching and organic matter decomposition [68]. Earlier research findings revealed higher mineral N under conventionally tilled soils but lower TN than conservation tillage [69]. Similarly, other authors confirmed that adopting minimum tillage usually increased soil total N (Govaerts et al., 2006b). On the contrary, Yagioka et al. [70] pointed out that minimum tillage reduced soil TN through leaching and volatilization.

Likewise, the cropping system was significantly affected the soil total N at Meskan but remarkable variation not observed at Hawassa Zuria. However, in both sites there was a tendency for better soil total N in the haricot bean-maize rotation system compared to maize monocropping (Table 6). The findings of this study agree with those of Kirkegaard et al. [71], who discovered that cereals can benefit from legume-based rotation systems not only in terms of yield but also in terms of soil total nitrogen, as opposed to cereal monoculture. Because legume residues had greater quality and a narrow C:N ratio, which resulted in the quick release of N from the residues, the legume-cereal-based rotation system provided more organic N than monocropping [46]. The effect of cropping systems on soil total N is not comparable in the two experimental sites/soils, possibly due to the differences in soil properties as indicated by Giller [72].

The use of nitrogen fertilizers had a considerable impact on soil TN. The combined application of compost and inorganic nitrogen fertilizer significantly enhanced the soil TN contents at both sites (Table 6). The integrated N-treatment had the highest TN (0.26% and 0.39% for Hawassa Zuria and Meskan, respectively), indicating that more N was released through mineralization of the compost added to the soil and due to the existence of high levels of respective total N in the compost. Our findings are in line with those of Ashenafi et al. [60] and Yan et al. [73], who found that inorganic nitrogen influences most soil biological processes by promoting microbial carbon use, which is critical for mineralization and nutrient transformation activities. In brief, total nitrogen status in soils showed a better response to the combined application of inorganic N fertilizer with compost than sole inorganic fertilizer.

In both sites, tillage methods had a significant impact on the C:N ratio, with minimum tillage giving a lower C:N ratio than the conventional tillage (Table 6). The lower C:N ratio in soils cultivated with minimum tillage may help to slow down the loss of nitrogen during organic matter decomposition. Similarly, cropping systems had a considerable impact on the C:N ratio at Meskan but not at Hawassa Zuria. However, when compared to maize monocropping, the legume-maize rotation system had a lower C:N ratio in both locations. The lower C:N ratio in soils treated with the

TABLE 7: Pearson's correlation coefficients for NDVI, grain yield, N-content, N-uptake, and grain protein content of maize grown with two tillage methods, two cropping systems, and four nitrogen levels at Hawassa Zuria (upper right side) and Meskan (lower left side).

	NDVI	GY	GNC	GNU	GPC
NDVI	1	0.59*	0.32 <sup>ns</sup>	0.46 <sup>ns</sup>	0.33 <sup>ns</sup>
GY	0.84***	1	0.71***	0.89***	0.71**
GNC	0.73**	0.73**	1	0.95***	0.99***
GNU	0.85***	0.95***	0.90***	1	0.95***
GPC	0.74**	0.74**	0.99***	0.91***	1

Significant at \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ; ns: not significant. NDVI: normalized vegetation index; GY: grain yield; GNC: grain N-content; GNU: grain nitrogen uptake; GPC: grain protein content.

haricot bean-maize rotation system could contribute to the higher nitrogen availability due to rapid N release from the residues and N-fixation [46]. The effect of N-fertilization on the C:N ratio was highly significant ( $P < 0.001$ ), and the narrow value was obtained in the integrated N-treatment, followed by sole inorganic N fertilizer (Table 6). This signifies that there was better mineralization of N from the applied compost. This finding was in line with Mamuye et al. [74], who found that combining organic and inorganic N-sources enhanced the C:N ratio significantly more than using either organic or chemical N-inputs alone.

**3.8. Pearson's Correlation Coefficients.** Pearson's correlation coefficient of grain yield was positively and significantly associated with NDVI, grain N-content, N-uptake, and grain protein content ( $r = 0.59, 0.71, 0.89,$  and  $0.71$ ) in Hawassa Zuria and Meskan ( $r = 0.84, 0.73, 0.95,$  and  $0.74$ ). These results are consistent with the findings of Simić et al. [39] who indicated that grain yield was positively and significantly associated with grain protein content ( $r = 0.82$ ) and nitrogen uptake. NDVI reading was positively and significantly correlated with grain yield ( $r = 0.59$ ) at Hawassa Zuria (Table 7), whereas, in Meskan, NDVI was positively and highly associated with grain yield, N-content, N-uptake, and grain protein content ( $r = 0.84, 0.73, 0.85,$  and  $0.74$ ), suggesting that NDVI reading is useful for acquiring information such as photosynthetic efficiency and potential yield indirectly [21].

## 4. Conclusions

Soil management practices significantly affect grain yield, N-content, N-uptake, and grain protein content and selected soil chemical properties. In both sites, the conventional tillage and rotation system increased the grain yield, N-content, N-uptake, and protein content in contrast to the minimum tillage and monocropping, respectively. Similarly, nitrogen fertilization clearly affected the grain yield, N-content, and N-uptake with the addition of  $92 \text{ kg N ha}^{-1}$  and  $46 \text{ kg N ha}^{-1} + 10 \text{ t compost ha}^{-1}$  treatments beating at Hawassa Zuria and Meskan sites, respectively. However, tillage methods and N-fertilization differed in their effects on soil chemical properties, and the MT and integrated

N-treatment improved soil organic carbon and total nitrogen concentrations compared to CT and other N-treatments, respectively. Grain yield was positively and significantly associated with NDVI, grain N-content, N-uptake, and protein content. Therefore, a conventional tillage plus haricot bean-maize rotation system with the addition of solely 92 kg N ha<sup>-1</sup> and integrated 46 kg N ha<sup>-1</sup> + 10 t compost ha<sup>-1</sup> could be recommended for Hawassa Zuria (Cambisols) and Meskan (Chernozem) districts, respectively, in order to achieve better yield and N-uptake. However, in order to ensure sustainable maize production in the studied sites, we concluded that the integrated N-treatment along with minimum tillage and legume-based crop rotation could enhance soil properties and will improve yields and N-uptake.

### Data Availability

The data are already included in the manuscript.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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