Abundance of native rhizobia nodulating cowpea in major production areas of Ethiopia as influenced by cropping history and soil properties

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ABSTRACT

Enumeration of the native rhizobia population is important to determine the abundance of rhizobia in the soil and the achievement of inoculation. In Ethiopia, limited information is available on the population abundance of cowpea nodulating rhizobia. This study aimed to evaluate the population abundance of rhizobia nodulating cowpea and their relation with cropping history and soil properties in cowpea producing areas of Ethiopia. The abundance of rhizobia existing in the soils was assessed by the most probable number technique. The study revealed that the population abundance of rhizobia nodulating cowpea is high, ranging from $3.1 \times 10^4$ to $1.0 \times 10^7$ rhizobia cells g$^{-1}$ of soil, and the population varied at each location. Besides, there was no statistically significant correlation between soil physicochemical properties and the rhizobial population. All the investigated soils had been cropped with cowpea in monoculture (sole cowpea), intercropping (mostly with sorghum and maize) and crop rotation for many years. Thus, the higher rhizobia population observed in this study is associated with the season factor and cropping history of the areas. In general, the soils of cowpea production areas in Ethiopia harbor adequate levels of rhizobia capable of nodulating cowpea, which are passable to provide satisfactory nitrogen fixation and nodulation.

Introduction

Loss of soil fertility is one of the most important constraints to legumes production (Kebede, 2020a; 2020b). Biological nitrogen fixation (BNF) technology is considered the best alternative to resolve this problem, especially under smallholder circumstances in the emerging countries where cowpea is grown without the use of fertilizers (Timko et al., 2007). However, for BNF to be efficient in fixing nitrogen as well as increasing crop yield, the population size of native rhizobia plays a significant role (Lindstrom et al., 2010). Native rhizobia are imperative in legume BNF to enhance the growth and yield of most legume crops, especially where inoculants are inaccessible (Woomer et al., 1997).

Cowpea (*Vigna unguiculata* L.) is the most important component of tropical agricultural systems due to its capability to recover marginal lands through nitrogen fixation and as a cover crop (Kebede & Bekeko, 2020). It is considered promiscuous in its association with root nodule-dwelling bacteria, so-called rhizobia. Rhizobia associated with cowpea are also of special significance to nitrogen fixation because they can form nodules on a wide range of tropical legumes. In optimal environments, well-nodulated cowpea can obtain 90% of their nitrogen required for maximum yield from an effective symbiosis (Eaglesham et al., 1977). Therefore, the use of cowpea as an element of cropping systems offers the biologically fixed nitrogen as a key factor in low input agricultural systems to withstand long-standing soil fertility.

The size of the native rhizobia population is the most prevailing ecological factor that regulates the competitive success of inoculated rhizobia (Thies et al., 1992). It has been found that the possibility of a response to inoculation with rhizobia strains diminished as the number of native rhizobia increased (Thies et al., 1991). Introduced rhizobia strains (inoculants) are continuously outcompeted with the native rhizobia strains, thus, it is not likely to improve BNF when native rhizobia population are beyond the threshold ($10^2$ rhizobia cells per gram of soil) and had some effective strains (Brockwell et al., 1995; Danso, 1992; Ereso, 2017; Thies...
et al., 1991). Besides, a variety of biotic and abiotic factors such as host plant, cropping history, soil pH, salinity, nutrient availability, soil organic carbon content, and texture are known to affect rhizobial population and diversity (Giller, 2001; Grönnemeyer et al., 2014; Law et al., 2007). It is, thus, crucial to know their geographical and ecological distribution and physicochemical soil necessities as differences in strain existence and profusion depend on these ecological parameters.

Information on the native rhizobial population is important for understanding the distribution and diversity of the rhizobia population in the soil and to determine the achievement of inoculation (Fening & Danso, 2002). Blazinkove et al. (2007) indicated that a study on the determination of the native rhizobial population plays a vital role in a better understanding of soil biodiversity and in improving the contribution of biologically fixed nitrogen to legume production. The evaluation of population and diversity within a native rhizobial population is also important for the screening of new and highly effective inoculant strains. Despite the cultivation of cowpea in Ethiopia, limited information is available on the native soil rhizobia that associate with cowpea (Kebede et al., 2021). Essentially, information linked to the population abundance of rhizobia, cropping history and physicochemical properties of the soil is very rare. Therefore, this work aimed to evaluate the size of the native population abundance of rhizobia-nodulating cowpea isolated from selected major growing areas of Ethiopia and to assess how their abundance relates to cropping systems as well as physicochemical soil parameters at the collection sites.

**Materials and methods**

**Description of soil collection area and soil sampling**

The soil samples for the study were collected from 10 selected districts of Oromia (five districts), Southern Nations, Nationalities and Peoples (SNNP) (three districts) and the Gambella region (two districts) (Table 1) (the location and map of each Kebele is shown in Figure 1). The sampling sites were selected in consultation with agricultural extension officers who knew farmers who grew cowpea and were willing to allow soil sampling from their fields. The soil samples were taken during the 2017/2018 cropping season from farmers’ fields which had no history of rhizobial inoculation by the time the cowpea crop was still growing in the fields. The samples were collected randomly from three farmers’ fields from each district using a zigzag pattern from the soil surface to a depth of 20 cm. All the collected soil

| Table 1. Description (region, Woreda, Kebele and sampling sites), coordinates, and cropping history of the soil collection sites |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Region          | Zone            | Woredas          | Kebeles         | Sampling Sites | Altitude (m)   | Latitude     | Longitude     | 2018            | 2017            |
|                 |                 |                  |                 |                 | (masl)         |             |              | 2016            | 2015            | 2014            |
| SNNPR           | South           | South Ari        | Kayisa          | 1               | 1387           | 0° 51' 26" | 36° 44' 04" | C M S+C         | CB T            |
|                 |                 |                  |                 | 2               | 1378           | 0° 31' 71" | 36° 37' 90" | C M+C S         | C S M           |
|                 |                 |                  |                 | 3               | 1362           | 0° 51' 92" | 36° 37' 97" | C C S C         | C C S           |
|                 |                 |                  |                 | 1               | 1148           | 0° 14' 31" | 37° 31' 24" | C C C C         | C C C           |
|                 |                 |                  |                 | 2               | 1167           | 0° 14' 46" | 37° 31' 29" | C C C C         | C C C           |
|                 |                 |                  |                 | 3               | 1162           | 0° 14' 27" | 37° 30' 71" | C C S C         | S C C           |
|                 |                 |                  |                 | 1               | 1390           | 0° 39' 51" | 37° 50' 07" | C M C S         | S M            |
|                 |                 |                  |                 | 2               | 2148           | 0° 39' 09" | 37° 49' 55" | T C F            | F               |
|                 |                 |                  |                 | 3               | 1383           | 0° 39' 13" | 37° 48' 52" | C M T C         | M               |
|                 |                 |                  |                 | 1               | 436            | 0° 14' 43" | 34° 29' 49" | C C C C         | C C C           |
|                 |                 |                  |                 | 2               | 415            | 0° 14' 42" | 34° 29' 44" | C C C C         | C C C           |
|                 |                 |                  |                 | 3               | 437            | 0° 14' 40" | 34° 29' 47" | C C C C         | C C C           |
|                 |                 |                  |                 | 1               | 446            | 0° 53' 17" | 34° 34' 32" | C C G S         | P               |
|                 |                 |                  |                 | 2               | 458            | 0° 53' 23" | 34° 34' 43" | C O C M         | C               |
|                 |                 |                  |                 | 3               | 468            | 0° 52' 99" | 34° 34' 11" | C C C C         | C C C           |
|                 |                 |                  |                 | 1               | 1906           | 0° 9' 19" | 42° 25' 41" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 2               | 1912           | 0° 9' 19" | 42° 25' 36" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 3               | 1904           | 0° 9' 34" | 42° 25' 34" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 1               | 1730           | 0° 14' 88" | 42° 18' 53" | S+C M+C G       | G S+C M+C     |
|                 |                 |                  |                 | 2               | 1633           | 0° 14' 04" | 42° 16' 96" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 3               | 1642           | 0° 14' 06" | 42° 19' 01" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 1               | 1671           | 0° 8' 54" | 40° 43' 06" | K+C K+C K+C    | K+C K+C K+C   |
|                 |                 |                  |                 | 2               | 1709           | 0° 53' 67" | 40° 43' 27" | C M M+C CB      | C               |
|                 |                 |                  |                 | 3               | 1738           | 0° 53' 69" | 40° 43' 29" | K+C K+C K+C    | K+C K+C K+C   |
|                 |                 |                  |                 | 1               | 1435           | 0° 10' 91" | 40° 39' 60" | C C M C         | M               |
|                 |                 |                  |                 | 2               | 1474           | 0° 10' 29" | 40° 40' 50" | C S C C         | S               |
|                 |                 |                  |                 | 3               | 1464           | 0° 10' 51" | 40° 40' 85" | C C M C M+C     | M               |
|                 |                 |                  |                 | 1               | 1693           | 0° 31' 88" | 42° 01' 78" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 2               | 1704           | 0° 31' 65" | 42° 01' 17" | S+C S+C S+C    | S+C S+C S+C   |
|                 |                 |                  |                 | 3               | 1704           | 0° 31' 63" | 42° 01' 16" | S+C S+C S+C    | S+C S+C S+C   |

S = Sorghum; C = Cowpea; M = Maize; K = Khat; CB = Common bean; G = Groundnut; T = Teff; P = Pumpkin; S+C = Sorghum intercropped with cowpea; K+C = Khat intercropped with cowpea; M+C = Maize intercropped with cowpea; O = Okra; F = Fallow
samples were kept in plastic bags with their full information and transported to Haramaya University and stored temporarily in the Agronomy laboratory. Data concerning the cropping history of the collection fields were collected in consultation with the farmers (personal communication with farmers).

The samples were, then, divided into parts for the chemical and physical analyses and determination of the rhizobia population abundance. For the determination of the rhizobia population in the collected soil samples, the samples were thoroughly mixed and a representative sample was taken from the three samples collected from each district to form a composite sample for each district. These samples were stored at 4°C for the most probable number (MPN) assay experiment to assess the populations of native rhizobia present in each district’s soil.

**Soil physico-chemical analysis**

The collected soil samples were analyzed at Haramaya University Soil Chemistry Laboratory for texture, pH, electrical conductivity, total N and available P, cation exchange capacity (CEC) and soil organic carbon following standard procedures as indicated in Table 2. All the samples were air-dried and ground to pass through a 0.5 mm sieve for nitrogen and organic carbon analysis and a 2 mm sieve for other physicochemical properties analysis.

**Enumeration of rhizobial population abundance nodulating cowpea**

The populations of native rhizobia existing in the soils of the collection area which could nodulate cowpea were

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**Figure 1.** Geographical distribution of soil sampling areas (each colored dot in the map of Ethiopia indicate sampling district and the map of each district is indicated with the respective color outside the map of Ethiopia).
assessed by the most probable number (MPN), plant infection technique as indicated by Somasegaran and Hoben (1994). In this study, representative soil samples from the soil collection areas were used for the estimation of numbers of the native rhizobial population. As indicated by Maingi et al. (2006), the size of native populations of rhizobia may vary within short distances from field to field. This was put into consideration when making representative soil samples from the soil collection area in such a way that the soil samples were collected randomly from three farmers’ fields from every 10 districts using a zigzag pattern to ensure consistency of the process. From the three samples taken from each district, thoroughly mixed and homogenous samples were taken to form a composite sample of the field in every 10 districts forming a total of truly representative soils of 10 samples. Therefore, MPN assay was done for these soil samples to assess the populations of native rhizobia present in each district’s soil.

The seeds of cowpea variety (Bole) were surface sterilized with 95% ethanol and in 3% (v/v) solution of sodium hypochlorite and successively rinsed with sterilized distilled water and allowed to air dry. Three cowpea seeds were adequately grown in acid-treated and sterilized sand using plastic pots and later seedlings were thinned to one plant per pot in Haramaya University greenhouse condition. Serial tenfold dilution was prepared for the 10 representative soil samples by briefly diluting 10 g of soil in 90 mL sterilized distilled water; then, 1 ml of diluents was added in 9 mL of sterile water to get the second diluents and the same procedure followed up to tenth dilution step \(10^{-10}\). A 1 ml of each dilution \((10^{-1} - 10^{-10})\) was subsequently used to inoculate the cowpea seedlings grown in acid-treated and sterilized sand using plastic pots and one control pot following each group of inoculated pots was used with four replications. These un inoculated controls were used to check for sterile conditions.

After 5 weeks of planting, the roots of the seedlings were gently washed with tap water and nodulation was assessed. Nodulation was recorded as “+” for the presence of nodulation or “—” for no nodulation and the number of nodulated (+) plants (units) was recorded beside each dilution. The total number of nodulated units was obtained by summing up the nodulated units at each dilution level (Appendix Table A1). The number of rhizobia in each soil was determined using the most probable number (MPN) plant infection technique. The MPN was calculated from the most likely number \(m\) obtained from the MPN tables according to the formula:

\[
\text{MPN} = \frac{mxd}{v}
\]

Where:
- \(X\) = MPN per gram of soil
- \(m\) = Likely number from the MPN table for the lower dilution of the series (Somasegaran & Hoben, 1985)
- \(d\) = Lowest dilution (the first unit used in the tabulation)
- \(v\) = Volume of aliquot applied to plant

### Results and discussions

#### Soil physico-chemical properties

Soil description concerning the assessment of the fertility status of the soils of an area is an essential aspect in the context of sustainable farming production. Taking this into account, different soil physicochemical properties were analyzed for soils collected from different cowpea growing areas for rhizobia isolation and characterization.

Laboratory analysis of physicochemical properties of soil sample revealed that the textural class of the soil was loamy sand for soil collected from Ifa, Bakanisa, Naliya Segen, Pinkew, Kayisa, and Biyo Awale, sandy clay loam (Cobo Kire and Oda Kanani) and sandy loam (llalam and Abala Faracho) (Table 3). The likely reason for the difference in the textural classes of soil collected from the different areas may be differences in topography, slope gradient, and parent material. Abate et al. (2016) reported that variations in soil texture may be caused by the difference in parent material, topography, in-situ weathering, and translocation of clay.

The soils typically ranged from moderately acidic to moderately alkaline with pH ranging from 5.76 (Abala Faracho) to 8.20 (Naliya Segen) (Table 3) according to

### Table 2. Soil physicochemical properties and their methods of analysis

| S. N. | Soil properties | Methods of analysis |
|------|----------------|---------------------|
| 1.   | Texture        | Bouyoucos hydrometer method (Bouyoucos, 1962). |
| 2.   | pH             | pH meter potentiometer method (Rhoades, 1982). |
| 3.   | Total nitrogen | Kjeldahl method as described by Bremner and Mulvaney (1982). |
| 4.   | Available phosphorus | Subjected to Olsen et al. (1954) method. |
| 5.   | Electrical conductivity | Determined using the electrical conductivity meter. |
| 6.   | Cation Exchange Capacity | Ammonium acetate method following the manual prepared by Sertsu and Bekele (2000). |
| 7.   | Soil organic carbon | Determined following the manual prepared by Sertsu and Bekele (2000). |
Table 3. Selected physical and chemical properties of the collected soil samples

| Soil collection districts | Textural class | pH  | OC (%) | OM (%) | Total N (%) | CEC (cmol (+)/kg) | EC (ds/m) | Ava. P (mg/kg) |
|--------------------------|----------------|-----|--------|--------|-------------|-------------------|-----------|---------------|
| Kayisa                   | Loamy sand     | 5.85| 0.10   | 0.18   | 0.056       | 12.4              | 0.05     | 2.76          |
| Naliya Segen             | Loamy sand     | 8.2 | 0.07   | 0.11   | 0.028       | 19                | 0.07     | 26.18         |
| Abala Farako             | Sandy loam     | 5.76| 0.10   | 0.18   | 0.27        | 21                | 0.04     | 28.45         |
| Pinkew                   | Loamy sand     | 6.54| 0.07   | 0.12   | 0.28        | 7.8               | 0.05     | 24.46         |
| Cobo Kire                | Sandy clay loam| 6.52| 0.12   | 0.21   | 0.042       | 33.8              | 0.05     | 31.15         |
| Ilaam                    | Sandy loam     | 7.12| 0.08   | 0.13   | 0.24        | 8                 | 0.04     | 2.76          |
| Ifa                      | Loamy sand     | 7.56| 0.10   | 0.18   | 0.042       | 14                | 0.04     | 8.59          |
| Bakanisa                 | Loamy sand     | 6.31| 0.17   | 0.30   | 0.035       | 39.2              | 0.06     | 13.99         |
| Oda Keneni               | Sandy clay loam| 7.52| 0.13   | 0.23   | 0.34        | 40                | 0.07     | 9.56          |
| Biyo Awale               | Loamy sand     | 7.96| 0.07   | 0.12   | 0.056       | 15.8              | 0.05     | 30.61         |

Murphy (1968) classification (Table A2). The pH of all the soil in the collection area was above the critical pH value for crop production which is 4.5 (Fairhurst, 2012). High soil pH values (alkaline condition) may have resulted from the soil management practices such as high evapotranspiration and low precipitation, which decreases the loss of the base forming cations from the soil as a result of leaching. According to McCauley et al. (2009), soils having pH near-neutral to alkaline conditions are mainly due to the occurrence of base-forming cations related to carbonates and bicarbonates found naturally in soils and irrigation waters, i.e. there is little leaching of base-forming cations due to comparatively low precipitation amounts, resulting in pH values greater than 7. Martyniuk and Oron (2008) reported that soil pH below 5.5 hinders nodulation and nitrogen fixation and is considered challenging for most microbial activities, and directly impacts the availability of nutrients to plants; thus, it does not favor the production of crops. Accordingly, the result of pH in all the collection areas was above the range which is indicated by these authors, thus, favorable for cowpea production and nitrogen fixation.

Total nitrogen (N) contents of the soil collection area ranged from 0.028% (Naliya Segen) to 0.34% (Oda Kanani) (Table 3). According to Tekalign (1991) ratings, the total N content of the studied soils is characterized under very low to high N content ranges. Accordingly, soil collected from Ifa, Cobo Kire, Bakanisa, and Naliya Segen had very low total N content and soil from Kayisa and Biyo Awale had low total N content which is 0.056% (Table A2). The low total N contents indicate that the soils of the study area are scarce in N to support appropriate growth and development of crops for expressing their yield potential, which suggests that the soils need fertilization with external N inputs and accumulation of their organic matter. The observed nitrogen deficiency in these soil samples could be due to low input of plant residues, low nitrogen-rich organic materials like manure and compost in agricultural systems. Abate et al. (2014) reported that the low total nitrogen content of the soil might be due to low organic matter content and low nitrogen release from the organic matter sources since soil nitrogen is certainly correlated with soil organic matter content. Soils collected from Ilaam (East Hararge) showed moderate total nitrogen content whereas soils collected from Pinkew, Oda Kanani, and Abala Farach showed high total nitrogen contents. As described by Okalebo et al. (2002), high nitrogen values above the critical limit of 0.25% reduce nodulation and nitrogen fixation. In this regard, the result of total nitrogen content in all collection areas was below the level that limits nodulation and fixation except soil collected from Pinkew (0.28% total nitrogen), Abala Farach (0.27% total nitrogen) and Oda Kanani (0.34% total nitrogen) which is rated as high.

The available phosphorus in the soils of the collection areas ranged from 2.76 (Ilam and Kayisa) to 31.15 mg kg soil⁻¹ (Cobo Kire) (Table 3). Based on Olsen et al. (1954) rating, soils collected from Cobo Kire, Bakanisa, Naliya Segen, Pinkew, Biyo Awale, and Abala Farach had highly available phosphorus content whereas Oda Kanani and Ifa had medium available phosphorus content (Table A2). Soils collected from Kayisa and Ilaam, however, had low available phosphorus contents. The higher available phosphorus contents in most soils of the collection area (60%) could be more likely due to the application of fertilizer (residual phosphorus), conducive soil pH for phosphorus accessibility and the related increase in a microbial action. The variability in available phosphorus contents of soils might be due to different soil management practices, specifically, the type and rate of organic fertilizers and inorganic fertilizer applied to the soil. According to Abate et al. (2016), variations in parent material, soil texture, degree of phosphorus fixation, soil pH and slope gradient may also contribute to variations in available phosphorus contents amid the land units.

The electrical conductivity of the soils ranged between 0.04 and 0.07 ds/m for all soil collection areas (Table 3) showing that there was no salinity problem in
the soil collection area (Herrera, 2005). The observed low EC value in all the soil collection areas in the present study designates a non-saline condition despite limited rainfall to leach away base-forming cations from the surface soil in the soil collection area according to Negash and Mohammed (2014).

The organic carbon and organic matter contents of the soils ranged between 0.07% to 0.17% and 0.11% to 0.30%, respectively (Table 3) and were very low according to Tekalign (1991) rating (Table A2). The low carbon and organic matter contents of the soils are characteristic of soils where the high rate of mineralization due to high temperatures decreases the buildup of carbon and organic matter. This higher depletion of soil organic carbon and organic matter contents may also be accredited to the fact that cultivation increases soil aeration which improves breakdowns of soil organic matter and most of the soil organic matters found in the cultivated soils are removed with harvest causing for its decrement. The lower organic matter content of these soils with comparatively low to higher available P content also indicates a quicker rate of organic matter breakdown and mineralization as reported by Abate et al. (2016). Yitbarek et al. (2013) in their study at Abobo (Gambella) indicated that soil OM content of cultivated soil is exhausted due to intensive cultivation, increased oxidation and whole removal of crop residues. Negash and Mohammed (2014) also revealed that low OM contents are accredited to a high temperature of the area that enhances the rate of organic matter decomposition, continuous cultivation with the whole removal of crop residue, limited application of farmyard manure and zero crop rotation.

The cation exchange capacity (CEC) of the soils ranged from 8 to 40 cmol (+)/kg (Table 3). Pinkew and Ilalam had low CEC based on Hazelon and Murphy (2007) rating (Table A2). Soil collected from Ifa, Naliya Segen, Kayisa, Biyo Awale, and Abala Faracho showed medium CEC, while Cobo Kire, Bakanisa, and Oda Kanani had high CEC. The difference in CEC values of the studied soils may be due to variation in organic matter content, type and amount of clay, and intensity of cultivation. Intensive cultivation reduces the CEC under the cultivated land as reported by Gao and Change (1996) and Abebe (1998).

**Abundance of native rhizobia nodulating cowpea**

The enumeration of rhizobia is valuable for the assessment of rhizobial population abundance in the soil and how they vary or to assess the number and viability of rhizobia in the soil (Howieson & Dilworth, 2016). The enumeration study revealed that the population size of native rhizobia compatible with cowpea crop is high and varied at various locations. The most probable number results revealed that the population size of the cowpea native rhizobia in the whole study area which are viable and infective ranged from $3.1 \times 10^4$ to $1.0 \times 10^7$ rhizobia cells $g^{-1}$ of soil (Table 4).

The lowest native rhizobial population was observed in the soils collected from Oda Kanani (Mieszio) while the highest population was observed in Pinkew (Gambella). Soil collected from Oda Kanani showed the lowest rhizobia cells $g^{-1}$ of soil which could be attributed to high nitrogen content (0.34% total N) as described by Okalebo et al. (2002) who reported that high nitrogen values above 0.25% suppress nodulation. However, soils collected from Pinkew and Abala Faracho had high nitrogen (0.28% and 0.27% total N, respectively) and ranked on 1st and 7th among the collection areas having a higher rhizobial population of $1.0 \times 10^7$ and $1.7 \times 10^6$, respectively (Table 4). This indicates that the amount of N content in these soils do not likely affect the nodulation. Even though cowpea is the most effective host plant of rhizobia (Kebede et al., 2020), this result showed that the symbiosis fixes nitrogen even if the soil nitrogen is adequate to meet the nitrogen demand of the crop in the case of Oda Kanani, Pinkew and Abala Faracho. On the other hand, low levels of available nitrogen content in the soils have little impact on nodulation and rhizobial population as soils having low and very low total N content showed higher rhizobial population abundance.

The study areas had a higher rhizobia population and none of the whole study areas had a low rhizobia population size (<$10^2$ rhizobia cells $g^{-1}$ of soil). Ahmad et al. (1981) reported cowpea rhizobia populations of $4.9 \times 10^7$, $3.5 \times 10^5$ and $4.3 \times 10^4$ cells $g^{-1}$ of soil which documented from three soils in West Africa. The result also showed that the levels of cowpea rhizobial population recorded in all study sites were adequate to give satisfactory outputs on nodulation and nitrogen fixation without inoculation. This is in agreement with the findings by Nambiar et al. (1983) who reported that most

| S. N | Soil collection districts | MPN (cells $g^{-1}$ of soil) |
|------|--------------------------|----------------------------|
| 1    | Kayisa                   | $1.0 \times 10^5$          |
| 2    | Naliya Segen             | $1.7 \times 10^6$          |
| 3    | Abala Faracho            | $1.7 \times 10^5$          |
| 4    | Pinkew                   | $1.0 \times 10^5$          |
| 5    | Cobo Kire                | $3.1 \times 10^5$          |
| 6    | Ilalam                   | $5.8 \times 10^5$          |
| 7    | Ifa                       | $1.0 \times 10^6$          |
| 8    | Bakanisa                 | $3.1 \times 10^6$          |
| 9    | Oda Kenenzi              | $3.1 \times 10^5$          |
| 10   | Biyo Awale               | $5.8 \times 10^5$          |
cultivated tropical soils have a rhizobial population of more than 100 rhizobia cells per gram of soil and are capable of nodulating the legumes grown on such soils. Fening and Danso (2002) also reported that estimates of the native *Bradyrhizobium* population capable of nodulating cowpea showed that most of the soils in Ghana contain large populations that can nodulate cowpea, with the highest numbers occurring in areas where cowpea is commonly cultivated.

Although the population of native rhizobia is higher, variations occur in the population size of the study site. Similar population size variations have been reported by Woomer et al. (1988), Thies et al. (1991), and Singleton et al. (1992). In Zimbabwe, Mpepereki (1994) reported population sizes of *Bradyrhizobium* species ranging from 0 to $2.9 \times 10^5$ cells g$^{-1}$ soil. From a wider perspective, Singleton et al. (1992) reported similar variability in cowpea rhizobial populations assessed by MPN when compared to values obtained from 305 tropical soil samples collected from locations around the world.

**Rhizobial abundance and cropping history of the sampled fields**

Cowpea is the main crop during the cropping seasons in most areas which leads to the presence and high population of cowpea as a host of rhizobia. In other words, the higher rhizobial population observed in this study may be associated with season factor and cropping history since the soil samples for MPN assay were collected during the main cropping season while cowpea is in the farmers’ field. According to the farmers’ responses during the soil samples collection regarding the previous cropping system, all the investigated soils had been cropped with cowpea either in monoculture (sole cowpea), intercropping (mostly with sorghum and maize) or crop rotation for many years (Farmers, personal communication). This reveals that the presence of an appropriate host is the major determinant of the presence and number of rhizobia. Chemining’wa et al. (2011) indicated that high population levels of cowpea nodulating native rhizobia in the field sites could be attributed to the legumes’ widespread integration of the cropping system in the Central Kenyan soils. Amarger (2001) further reported that rhizobia are widespread in tropical soil as a result of the natural distribution and cultivation of legumes showing a close relationship exists between legumes and the occurrence and rhizobial population.

Besides, the use of legume inoculants is not common in Ethiopia and is non-existent in the areas where the soils used in this study were collected, further indicating that the high rhizobial populations encountered in these soils are due to the long-established native population as a result of cowpea cultivation. Therefore, higher numbers of rhizobia that have previously supported the growth of host cowpea have been attributed to the high rate of nodulation and rhizobial multiplication in the host rhizosphere in this study. Using five different legume species, Woomer et al. (1988) confirmed that the importance of the appropriate host legume on the occurrence and proliferation of a population of a particular rhizobial species in soil. Martins et al. (2003) also reported that an increase in the rhizobia population from 10 cells to $10^2$ cells per gram of soil occurred after the first introduction of cowpea and another increase to around $10^4$ cells per gram of soil after the second crop. A seasonal increase in the rhizobia population in the cowpea trials and stimulation of the multiplication of rhizobia by cropping cowpea was also reported by Mulongoy and Ayanaba (1986) while studying seasonal rhizobia populations in three locations in West Africa. The report of Hegde (1994) and Thies et al. (1995) on marked enrichment of rhizobia following the cultivation of homologous legume and the influence of cropping systems also supports this finding and has brought into the better focus of the positive and negative impacts of the preceding crops.

On the other hand, the higher abundance and variation of rhizobia in the areas demonstrated that the existence of non-legume plants could also help the rhizobia to maintain their population at a higher level. Thus, the population and abundance of rhizobia are determined not only by the host legume cultivation history but also by other crops in rotation or intercropping system, fertilizer application, and crop management. Yan et al. (2014) reported that the reasons for the variation and diversity of rhizobial populations may be due to the different land-use histories, rotation or monoculture of the host plant, different fertilizer supplements, and planting of legume or nonlegume plants. The probable reason may be that different land use or crop management changed the soil conditions, making it favorable to support a higher diversity of rhizobia in the soil, subsequently affecting and proliferating the rhizobial population abundance. Yan et al. (2014) also showed that the possible association of rhizobia as endophytes of the other plants in the grassland and maize/wheat in the cropland and plants also have a preference for their endophytes, which in turn affected the diversity and abundance of rhizobia. Endophytic rhizobia initially colonize the root-cap surface, with many rhizobia...
remaining either attached to cells that have been sloughed off (Chaintreuil et al., 2000) or in the rhizoplane (Schloter et al., 1997).

In addition to the cropping system, the previous reports indicated that the diversity of Rhizobium population nodulating legumes was found to increase under water-limited conditions (Krasova-Wade et al., 2003). In line with this, the higher abundance of this cowpea rhizobial population may also be attributed to low rainfall in the soil collection areas as drier soils may be richer in rhizobia as reported previously. The higher richness of root nodule symbionts has already been reported for cowpea from low-rainfall areas in South Africa and Botswana by Law et al. (2007) who showed that cowpea rhizobia are more diversified in low rainfall areas. Grönemeyer et al. (2014) also observed that the symbiotic communities of rhizobia from semi-arid sampling sites were more diverse than such from humid sites in Namibia. Similarly, Krasova-Wade et al. (2014) reported a higher richness of cowpea-nodulating *Bradyrhizobium* strains from the drier north than the more humid south of Senegal. Ndungu et al. (2018) reported the reason as plant selectivity in association with rhizobia may be lower under drought than humid conditions. Rhizobial populations in tropical soils are also reported to represent an important reservoir from which superior strains adapted to environmental stresses such as drought can be selected (Mpepereki et al., 1997).

**Nodulation and inoculation success using native rhizobial abundance**

The population size of native soil rhizobia is a reliable index for the capacity of a legume crop to derive nitrogen through biological nitrogen fixation and to determine whether or not the legume will respond to inoculated rhizobia (Thies et al., 1991). The number of rhizobia present in the soil, their effectiveness, and their often-superior ability to compete with an inoculant strain determine the success of the inoculum. As indicated in Ereso (2017) report, the number of rhizobia needed for prompt nodulation lies between $10^2$ and $10^3$ cells g$^{-1}$ of soil.

In the present study, inoculation may not be necessary for all soil sample collection areas of this study. This is due to the fact that the soils of major cowpea growing areas of Ethiopia had a higher number of native rhizobia ($>10^3$ rhizobia cells g$^{-1}$ of soil) than what has been reported previously, indicating that the soils harbor adequate levels of rhizobia capable of nodulating, adequate to give satisfactory nitrogen fixation and nodulation on cowpea. The results may also reveal that the area may give negative responses to the rhizobial inoculants if the population size of native rhizobia available in the soil is the only factor affecting inoculant responses. Thies et al. (1991) and Brockwell et al. (1995) indicated that the response to inoculation and competitive success of rhizobia inoculant is inversely related to the number of native rhizobia. Moreover, in soils where native rhizobial populations are high ($>10^5$ rhizobia per g soil), the introduction of new strains can be difficult and often ineffective. Danso (1992) also reported that the population range of $10^3$ to $10^4$ rhizobia per gram of soil was by most standards adequate for nodulation to occur in food legumes.

Previous studies have also revealed that the population size of effective native soil rhizobia can be used as a reliable index of whether a legume would respond to inoculation or not. Thies et al. (1991) showed that inoculation of eight leguminous crops with commercial strains increased the number of nodules per plant only in soils containing 10 to 100 native rhizobial cells g$^{-1}$ soil. Therefore, it is usually difficult to introduce superior strains by inoculation when there are a large resident population and well-adapted rhizobia such as in this study.

**Relationship of rhizobial abundance and soil properties**

Indeed, the soil conditions and specific affinity between the rhizobia and host plants can determine the community structure and population abundance of the rhizobia (Yan et al., 2014). It is known that the survival of rhizobia can be affected at soil pH values $<$5.5, with severe reductions in numbers at pH $<$4.5 (Martyniuk & Oron, 2008). In this study, the pH of the study area ranged from 5.76 (Abala Faracho) to 8.20 (Naliya Segen); therefore, it is unlikely to have markedly affected the infection, survival, persistence, multiplication, population and subsequent root colonization of the rhizobia (Brockwell et al., 1991). There was no significant correlation between all soil factors (pH, total N, and available P) and the number of rhizobia populations harbored in tested soils. However, a positive correlation was noted between soil properties such as pH, total N and available P and number of the rhizobial population, while a negative correlation was observed with soil OC, OM, CEC and EC and rhizobial population (Table 5). Since there is no history of rhizobial inoculation at the sites, the populations identified were considered to be native strains with great adaptability to those regions, which could be the reason why there is no significant correlation between the rhizobial population and soil physicochemical properties. Mwenda et al. (2011) also reported a positive correlation between rhizobia diversity and soil pH and attributed high rhizobia diversity to cropping history due to unexpectedly high diversity of
rhizobia in soils from one of the sites with acidic pH. However, Woomer et al. (1988) reported a negative correlation between soil pH and root nodule bacteria indicating that acid soils are beneficial for the proliferation and survival of these root nodule bacteria.

As indicated in Table 5, the relationship between all soil properties and the native rhizobial population is not statistically significant. Musiyiwa et al. (2005) indicated that the population abundance of rhizobia was poorly correlated with soil physicochemical properties. This might be because all the soils analyzed had soil physicochemical properties that were favorable for rhizobia persistence and growth (Giller, 2001). A possible explanation for this result could thus be that the rhizobial population might not be influenced primarily by soil characteristics, likely indicating that populations of cowpea rhizobia in soil strongly depend on the cultivation of their host plant. Venkateswarlu et al. (1997) also reported that crop-related factors have a more critical influence on the abundance of native rhizobial populations than soil or climatic factors.

When assessing whether or not inoculation is required, in general, cowpea farmers should consider not only factors related to rhizobial population size and its effectiveness but also various environmental factors as well as agricultural practices as they may contribute to field dominance by the native strains. This study advocates that intensive studies across all seasons and areas are required in order to establish a measure of the size of the native rhizobia population in the soil, due to the reason that rhizobia do vary unevenly from place to place and even two soils within farms may have a very huge difference in numbers of rhizobia in it.

### Conclusions

The abundance of the native rhizobia population is the most predominant factor that regulates the success of inoculation. Thus, the determination of the native rhizobial abundance plays a vital role in understanding the distribution and diversity of the rhizobia population in the soil and in determining the achievement of inoculation. In this study, the enumeration study of the native rhizobial abundance revealed that the population size of native rhizobia compatible with cowpea is high in cowpea-producing areas of Ethiopia and the population varied at various locations. The higher rhizobia population observed is associated with the season factor and cropping history of the areas. There was no statistically significant correlation between physicochemical properties and native rhizobial population indicating that populations of cowpea rhizobia in soil strongly depend on the cultivation of their host plant. Therefore, it is possible to conclude that the soils of major cowpea growing areas of Ethiopia had adequate levels of rhizobia capable of nodulating and adequate to give satisfactory nitrogen fixation and nodulation in cowpea production.

### Public interest statement

Rhizobia nodulating cowpea is of special significance, providing biologically fixed nitrogen as a key factor in low input agricultural systems. However, the population abundance of rhizobia in the soil determines the success of nitrogen fixation and is usually influenced by cropping history and soil properties. This article evaluated the population of rhizobia nodulating cowpea and their relation with cropping history and soil properties in Ethiopia. Accordingly, the study revealed that the population abundance of rhizobia existing in the soils of Ethiopia is high and varied across the location. The study also showed the relationship between the rhizobia population and cropping history and soil properties. Consequently, it is of paramount importance to determine the population abundance of rhizobia in the soil which could be a reliable index for the capacity of a legume crop to derive nitrogen through biological nitrogen fixation and the achievement of inoculation.

### Disclosure statement

The authors declare no competing interests.

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**Table 5. Correlation among soil physicochemical properties and population of rhizobia**

|       | MPN  | pH   | OC   | OM   | Total N | CEC  | EC   | Available P |
|-------|------|------|------|------|---------|------|------|-------------|
| MPN  | 1.000|      |      |      |         |      |      |             |
| pH   | 0.008| 1.000|      |      |         |      |      |             |
| OC   | 0.260| -0.381| 1.000|      |         |      |      |             |
| OM   | -0.234| -0.417| 0.996**| 1.000|         |      |      |             |
| Total N | 0.224| -0.168| -0.099| -0.090| 1.000 |      |      |             |
| CEC  | -0.367| -0.060| 0.838**| 0.820**| -0.032| 1.000|      |             |
| EC   | -0.187| 0.391| 0.268| 0.226| -0.046| 0.575| 1.000|             |
| Available P | 0.071| 0.094| -0.235| -0.237| -0.122| 0.128| 0.085| 1.000       |

*aCorrelation is significant at the 0.01 level.*
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## Appendixes

### Table A1. Nodulation of cowpea during MPN determination in the collected soils.

| Soil collection districts | Dilution levels | Nodulated units | MPN (cells g⁻¹ of soil) |
|---------------------------|-----------------|-----------------|------------------------|
|                           | $10^{-1}$       | $10^{-2}$       | $10^{-3}$   | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ |
| Kayisa                   | 4               | 4               | 3           | 3         | 2         | 2         | 1         | 0         | 0         | 0         | 19             | 1x10⁵         |
| Naliya Segen             | 4               | 4               | 4           | 3         | 3         | 2         | 0         | 0         | 0         | 0         | 24             | 3.1x10⁵       |
| Abala Faracho            | 4               | 4               | 4           | 3         | 3         | 2         | 2         | 1         | 0         | 0         | 0         | 20             | 3.1 x10⁶      |
| Pinkew                   | 4               | 4               | 4           | 4         | 4         | 4         | 2         | 1         | 0         | 0         | 0         | 27             | 1.7x10⁶       |
| Cobo Kire                | 4               | 4               | 4           | 3         | 3         | 2         | 2         | 1         | 0         | 0         | 0         | 21             | 1.0x10⁶       |
| Ilalam                   | 4               | 4               | 4           | 4         | 3         | 3         | 2         | 2         | 1         | 0         | 0         | 22             | 5.8x10⁵       |
| Ifa                      | 4               | 4               | 4           | 3         | 3         | 3         | 2         | 2         | 1         | 0         | 0         | 23             | 3.1x10⁶       |
| Bakanisa                 | 4               | 4               | 4           | 3         | 3         | 3         | 2         | 2         | 1         | 0         | 0         | 25             | 1.0x10⁶       |
| Oda Keneni               | 4               | 4               | 4           | 4         | 3         | 3         | 3         | 2         | 1         | 0         | 0         | 20             | 3.1x10⁵       |
| Biyo Awale               | 4               | 3               | 3           | 3         | 3         | 3         | 1         | 1         | 0         | 0         | 0         | 18             | 1.7x10⁵       |

### Table A2. Ratings used for determining the status of soil physicochemical properties collected from different sources.

| Rating | Avail P (mg kg⁻¹) | Rating | Total N (%) | Rating | OC (%) | OM (%) | Rating | pH | Rating | CEC cmolc kg⁻¹ |
|--------|-------------------|--------|-------------|--------|--------|--------|--------|-----|--------|---------------|
| Low    | < 5               | Very low| < 0.05     | Very low| < 0.50 | < 0.86 | Very strongly acidic | < 5.0 | Very low | < 6 |
| Medium | 5–10              | Low    | 0.05–0.12  | Low    | 0.5–1.5| 0.86–2.59| Strongly acidic | 5.1–5.5 | Low | 6–12 |
| High   | > 10              | Moderate| 0.12–0.25 | Moderate| 1.5–3.0| 2.59–5.17| Moderately acidic | 5.6–6.0 | Moderate | 12–25 |
| High   | 18–25             | High   | > 0.25     | High   | > 3.00 | > 5.17 | Slightly acidic | 6.1–6.5 | High | 25–40 |
| Very high | > 25              | Source: Tekalign (1991) | Very high | Not Given | Not Given | Neutral | 6.6–7.3 | Very high | > 40 |
| Very high | > 25              | Source: Olsen et al. (1954) | Tekalign (1991) | Source: Tekalign (1991) | Source: Tekalign (1991) | Slightly alkaline | 7.4–7.8 | Source: Hazelton and Murphy (2007) | Moderately alkaline | 7.9–8.4 |
| Very high | > 25              | Source: Murphy (1968) | Very strongly alkaline | 8.5–9.0 | Very strongly alkaline | > 9.0 | Source: Murphy (1968) | Very strongly alkaline | > 9.0 | Source: Murphy (1968) | Very strongly alkaline | > 9.0 |