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Farm and soil fertility management-induced shifts in arbuscular mycorrhizal fungi (AMF) population density and diversity under wheat and Teff croplands

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Abstract

Arbuscular mycorrhizal fungi (AMF) serve as vital elements of agricultural ecosystems, contributing significantly to enhanced crop yields and environmental stress tolerance. This research aimed to assess how various soil fertility management strategies changed the AMF community composition within teff and wheat croplands. Soil samples were obtained through random selection from specific districts, and spores recovered by employing the wet sieving and decantation technique. The results indicated that average spore density varied significantly ($p < 0.05$) across different sampling locations, districts, crop types, and between sampling and control groups. The Debre Elias district yielded the highest average AMF population, reaching 56.67 spores 100 g^{-1} dry soil. A similar peak in spore density was observed specifically in wheat-cultivated lands. Taxonomic analysis revealed 38 distinct AMF species/morphotypes categorized into three AMF genera: *Acaulospora, Glomus*, and *Pacispora*. Of these, *Acaulospora* was identified as the most prevalent genus, with *Acaulospora scrobiculata* and *Acaulospora myriocarpa* being the most frequently occurring species. These results underscore the profound impact of land management and soil fertility practices on AMF populations. Such dynamics are critical for optimizing nutrient cycling, strengthening climate resilience, and fostering long-term agricultural sustainability.

1. Introduction

Sustainable farm and soil fertility management activities like, reduced tillage, crop rotation, intercropping, organic amendments, as well as utilization of cover crops promote AMF colonization and diversity by maintaining soil structure and providing continuous host plants. In contrast, farm and soil fertility management practices like excessive chemical fertilizer and pesticide use, mono-cropping, and frequent soil disturbance (tillage) reduce AMF populations by disrupting hyphae networks and lowering plant dependence on fungal symbiosis [1].Therefore, integrating organic and biological inputs instead of relying solely on synthetic fertilizers helps to sustain arbuscular mycorrhizal fungi (AMF) activity, improving nutrient cycling, absorption and translocation of phosphorous (P) and water, soil fertility, and plant resilience [1].

AMF establish a symbiotic association with land plant roots. The symbiosis helps the AMF to complete life cycle [2, 3]. These fungi are among the most widespread and advantageous soil microbes found in both natural and agricultural environments, forming partnerships with more than 85% terrestrial plant families [2].

AMF enhance plant growth and development by supplying water, essential soil nutrients, especially phosphorus and nitrogen, and by offering resistance against pathogens [4]. In exchange, the fungi obtain photosynthetic products from the plant to support their growth and development. These fungi are obligate symbionts, which colonize the roots of plants and develop arbuscules, specialized structures within the root cortex cells that serve as key sites for nutrient exchange [2]. At the interface between soil and plant roots, the interactions between crop roots together with soil microbes result in diverse types interactions related to associations [5], which could affect the organisms involved in the interaction. In a mycorrhizal partnership, AMF help host plants cope with abiotic and biotic stressors and enhance plants absorption of inorganic nutrients [6]. This symbiosis also supports plants resilience the soil-borne pathogens, enhances the ability to withstand drought as well as promotes the development of a stable soil structure through aggregate formation by producing a glycoprotein called glomalin. AMF are also considered effective bio-indicators of soil health [7]. Through the production of glomalin and organic acids, AMF facilitate carbon sequestration, bind heavy metals, and minimize soil erosion by promoting soil aggregate formation and aid in soil mineralization by producing enzymes linked to organic phosphorus availability [8]. They serve a crucial role in the uptake and transport vital nutrients, by enhancing plant growth and productivity. The extensive network of AMF hyphae allows them to access a greater amount of soil beyond root depletion zone compared to non-mycorrhizal plant roots, efficiently absorbing nutrients like nitrogen, phosphorus, and potassium through osmotrophic mechanisms, which contributes to higher crop yields [4].

Although several research findings have indicated that the phytobeneficial effects of AMF in promoting plant nutrient absorption, water absorption, and tolerance to environmental stresses, there remains limited understanding about how

population density and species diversity of AMF vary under distinct farm and soil fertility management practices and cropping systems.. The impacts of farm and soil fertility management activities in AMF population density and species diversity under teff (*Eragrostis tef*) and wheat (*Triticum aestivum*) croplands in the East Gojam zone, northwestern, Ethiopia has not been thoroughly investigated. Therefore, we hypothesize that AMF population density and species diversity varied between teff and wheat croplands, between controls and samples and among sampling locations and districts. Thus, the present study aims to assess AMF population density and species diversity in teff, and wheat crop fields at selected districts in the East Gojam Zone, northwest, Ethiopia.

2. Materials and Methods

2.1 The study areas

The administrative center of these sampling districts is Debre Markos, which serves as a midpoint between two major hubs, situated approximately 299 km from Addis Ababa, and 265 km from Bahir Dar. The region's environmental and agricultural characteristics include: 1) Climate: an average annual precipitation of 1380 mm, with annual average minimum and maximum temperature in the area was 15°C and 22°C respectively. 2) Agriculture: The local economy is dominated by the cultivation of diverse crops, primarily teff, wheat, maize, and barley, alongside pulses such as beans, chickpeas, and lupine.

2.2 Selection of study districts, and farmlands

This study utilized a randomized selection process to identify specific districts and individual farmlands for sampling. Four districts—Dejen, Aneded, Awabel, and Debre Elias were chosen due to their status as high productivity hubs for teff and wheat within the region. Within each of these districts, four to five representative farmlands were selected for inclusion. To contextualize the soil environment, a preliminary survey was conducted using a structured data collection instrument (Supplementary Information 1). This survey focused on documenting the following management practices over the past ten year period: Cropping history: previous crop rotations and land use patterns; Nutrient management: historical application rates and frequencies of chemical fertilizers (P&N) and chemical inputs: the frequency and type of pesticide applications across the selected sites were assessed.

2.3 Soil sampling and transportation

We obtained soil samples from wheat and teff croplands across the selected districts between December 2023 and February 2024 (the dry season). A total of 20 sampling locations were established, including managed farmlands and adjacent untreated plots which served as controls. Sampling Protocol: surface vegetation and debris were cleared at each sampling point prior to collection. We collected triplicates of soil samples by digging a 20 cm depth. To ensure a representative sample, soil was collected from five points within each farmland and pooled into a one composite sample per farmland. Approximately 1 kg was obtained per farmland and the collected samples were secured in sterile container and taken to the Mycology Laboratory, Department of Biology at Debre Markos University, Following initial processing, samples were air-dried and carried to the Applied Microbiology Laboratory at Addis Ababa University for AMF spore recovery and further analysis. Each 1 kg soil was divided into two equal portions:500 g was utilized for the analysis of soil parameters and the remaining 500 g was reserved for AMF spore extraction.

2.4 Soil physicochemical analysis

Soil pH was measured following the protocol described in Gitari [10]. The specific procedure was as follows. Approximately 10 g of soil was put into a 60 ml flask and we added 20 ml of distilled water.. The suspension was mixed vigorously for 10 minutes and then left to equilibrate for 30 minutes. Readings were taken using a glass electrode pH meter, which was thoroughly rinsed with distilled water between subsequent samples to ensure accuracy. Additional soil chemical properties were determined using the following standardized methods. Soil organic carbon (SOC) was quantified

via the Walkley and Black chromic acid oxidation method [11]. Total nitrogen (TN) was determined by using the Kjeldahl digestion and distillation procedure [12]. Available phosphorus (P) was analyzed by using the Olsen extraction method [13].

2.5 Arbuscular mycorrhizal fungi spore extraction

The extraction of AMF spores from the collected soil was conducted using the wet sieving and decanting technique, as detailed by Gerdemann and Nicolson [14]. The procedure was performed in triplicate for each sampling location, utilizing a total of 300 g of air-dried soil (100 g per replicate). For each replicate, 100 g soil was thoroughly mixed into 500 ml of water and left to equilibrate for 1–2 hours. Any coarse soil aggregates were gently manually crushed. The suspension was passed through four sieves with decreasing mesh sizes arranged top to bottom: 500, 212, 90, and 45 µm. The washing cycle was continued until the filtrate ran transpararent. The residues on the 500 µm sieve were inspected for sporo-carps or spore clusters before being discarded. Materials retained on the 212 and 90 µm sieves were rinsed and consolidated onto the 45 µm sieve for further processing. The concentrated materials from the 45 µm sieve were transferred into 15 ml Falcon tubes and subjected to centrifugation at 2,000 rpm for 5 minutes. The supernatant was then discarded and the remaining pellets were re-suspended in a 50% (w/v) sucrose solution and centrifuged again at 2,000 rpm for 3 minutes. The resulting supernatant, containing the spores, was decanted back onto the 45 µm sieve and rinsed thoroughly with water to remove residual sucrose. Finally, the spores were transferred into 90 mm gridded plastic Petri dishes for morphological assessment and counting.

2. 6 AMF spore enumeration

Spore density was determined using a stereomicroscope (ISO1006) at 40× adhering to the standardized protocols established by the International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). To estimate the total spore population per sample, a systematic field-of-view calculation was employed. The surface area of the Petri dish base and the area of the microscopic field were calculated based on their respective diameters. The total number of potential microscopic fields within a single dish was derived using the following ratio: The total number of microscopic fields per dish was then determined using the formula: Total number of fields=Area of the dish/area of the lens. The total numbers of spores were counted across 40 randomly selected microscopic fields per dish. The average number of spores per field was then multiplied by the total number of fields to calculate the estimated spore density per Petri dish (representing 100 g of air-dried soil).

2.7 AMF identification

Taxonomic identification of AMF spores was conducted by a collaborative effort between the Addis Ababa University,Ethiopia and the Federal University of Minas Gerais, Brazil. Healthy, representative spores were isolated under a dissecting microscope using a micropipette. These specimens were permanently mounted on microscope slides using a 1:1 (v/v) polyvinyl-lactic acid-glycerol (PVLG) solution. To expose internal wall layers, the spores were carefully crushed and allowed to dry for 14 days at room temperature prior to detailed examination. Detailed morphological analysis was performed by using a compound microscope (Olympus BX51) at magnifications of 20×, 40×, and 100×. High-resolution images were captured via a fixed digital camera to document diagnostic features. The spores were identified to the genus and species level based on key morphological traits, including: Spore wall architectures like number, thickness, and arrangement of layers; surface features such as color and specific ornamentation were considered. In addition, subtending hyphae features like attachment characteristics and morphology were included. Identification was followed the standardized guidelines and reference given by the International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and the Glomeromycota database.

2.9 Statistical analysis

All statistical evaluations were carried out by using SAS (version 9.4). A one-way Analysis of Variance was employed to evaluate significant variations in average AMF spore densities across various sampling locations, districts, and crop types (wheat vs. teff). Where significant effects were observed, LSD was employed for average separation at $p < 0.05$. The AMF community structure within the study area was characterized using the following parameters: Spore density (SD): Defined as the total number of spores 100 g^{-1} dry soil. Species richness (S): Calculated as the total number of unique AMF species identified per sampling area. The Shannon-Wiener diversity index (H') was calculated using the formula: $H' = \sum \left(\frac{ni}{n}\right) \ln \frac{ni}{n}$, where ni is the number of individuals of a species and n is the total number of individuals of all species. Simpson's dominance index (D) was calculated as: $D = \sum \left(\frac{ni}{n}\right)^2$. Species richness (SR), isolation frequency (IF), and relative abundance (RA) were used to assess the structure of AMF communities. Isolation frequency (IF) was calculatedas: $\frac{\text{the number of samples in which a particular AMF species was observed}}{\text{the total number of samples}} X\ 100$

Importance Value (IV): Calculated as the average of the relative abundance and isolation frequency: IV= (IF + RA)/2, following the classification established by [15]. AMF species and genera were categorized based on their IV values. That is, if, IV > 50%, "dominant", if, 10 < IV < 50, "common" and if, IV < 10%, "Rare".

3. Results

3.1 The preliminary survey of farm and soil fertility management practices

The survey results exhibited that the soil types of the croplands included in this study were black and red soil types. All (100%) of teff cultivated croplands were black soils, which was about 76% of soil samples include in this study (Table 1). Among the sampled croplands soils, 29% were red soils with lime amendment history in 2020/2021 cropping season. Among these, 23% were wheat croplands. The previous cropping history of the croplands also exhibited crop rotation practices (Table 1).

Table 1
Results of preliminary survey

Sample number	District	Region	Soil type	Crop type	Previous crop	Year of liming	N fertilization	P fertilization	Pesticide application
1	Aneded	Amhara	black	Teff	Wheat	None	Yes, in the last ten years	Yes, in the last ten years	Yes
2	Aneded	Amhara	Black	Teff	Vetch	None	Yes, in the last ten years	Yes, in the last ten years	Yes
3	Aneded	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
4	Aneded	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
5	Awabel	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
6	Awabel	Amhara	Black	Teff	Wheat	None	Yes, in the last ten years	Yes, in the last ten years	Yes
7	Awabel	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
8	Awabel	Amhara	Black	Teff	Wheat	None	Yes, in the last ten years	Yes, in the last ten years	Yes
9	Debre Elias	Amhara	Red	Teff	Teff	2018/2019	Yes, in the last ten years	Yes, in the last ten years	Yes
10	Debre Elias	Amhara	Red	Wheat	Teff	2020/2021	Yes, in the last ten years	Yes, in the last ten years	Yes
11	Debre Elias	Amhara	Red	Wheat	Niger	2020/2021	Yes, in the last ten years	Yes, in the last ten years	Yes
12	Debre Elias	Amhara	Red	Wheat	Wheat	2020/2021	Yes, in the last ten years	Yes, in the last ten years	Yes
13	Debre Elias	Amhara	Red	Wheat	Teff	2020/2021	Yes, in the last ten years	Yes, in the last ten years	Yes
14	Dejen	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
15	Dejen	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes
16	Dejen	Amhara	Black	Teff	Niger	None	Yes, in the last ten years	Yes, in the last ten years	Yes

Sample number	District	Region	Soil type	Crop type	Previous crop	Year of liming	N fertilization	P fertilization	Pesticide application
17	Dejen	Amhara	Black	Teff	Teff	None	Yes, in the last ten years	Yes, in the last ten years	Yes

In general about 47% of the croplands included in this study exhibited crop rotation practices. About 75% of wheat croplands exhibited different crops previous year (2021/2022). Similarly, about 38% of the teff croplands experienced crop rotation practices including cereals and legumes (Vetch). The survey results further revealed that the farm and soil fertility management practices have been involving intensive chemical fertilizer and pesticide applications in all the sampling locations and districts in the past ten years (Table 1).

3.2 Soil physicochemical properties

The soil properties varied across the studied districts (Table 2). The soil pH was between 6.2 in Dejen district major teff grower to 7.1 in Debre Elias major wheat grower district. The organic carbon content was highest in Awabel district (3.2%) and lowest in Aneded district (2.1%), while organic matter content ranged from 3.6% to 5.5% across the sites. Similarly, total nitrogen and available phosphorus differed among the districts, with values ranging from 0.94% to 1.27% and 16.25 to 26.5 ppm, respectively (Table 2).

Table 2
Soil parameters in the districts

Districts	pH	Organic carbon (OC %)	Organic matter (OM %)	Total nitrogen (TN% %)	Available phosphorus (P, ppm)
Aneded	6.6	2.1	3.6	0.98	16.25
Awabel	6.8	3.2	5.5	1.26	18.7
Debre Elias	7.1	2.6	4.5	0.94	23.6
Dejen	6.2	3.1	5.3	1.27	26.5

3, **Arbuscular mycorrhizal fungi spore density at each district**

3.3.1 AMF spore density in Aneded district

The average AMF spore density per 100 g dry soil in the teff cropland varied significantly ($p < 0.05$) among the sampling locations in Aneded district. Figure 2 shows the average AMF spore density 100 g^{-1} dry soil across these locations.

The average AMF population density ranged between 46 spores 100 g^{-1} dry soil at sampling location two (S2) to 59 spores 100 g^{-1} at sampling location three (S3) (Fig. 2). In contrast, the control samples exhibited considerably a higher average AMF spore density of 115.33 spores 100 g^{-1} dry soil (Fig. 2).

3.3,2.AMF population density at Awabel district

The average AMF spore density 100 g^{-1} dry soil in the teff croplands varied significantly ($p < 0.05$) among the sampling locations in Awabel district. The highest average AMF spore density (48.5 spores 100 g^{-1} dry soil) was observed at sampling location four (S4), while the lowest (44.33 spores 100 g^{-1} dry soil) was recorded at sampling location one (S1) (Fig. 3).

Overall, average AMF spore density across the sampling locations ranged between 44.33 to 48.5 spores 100 g^{-1} dry soil under teff cultivation/ cropland (Fig. 3).

3.3.3 AMF population density at Debre Elias district

The average AMF spore density 100 g^{-1} dry soil varied significantly ($p < 0.05$) among sampling points in Debre Elias district under the wheat croplands. Average AMF spore density ranged between 47 spores 100 g^{-1} dry soil at sampling location three (S3) to 55 spores 100 g^{-1}at sampling location one (S1). In contrast, the control samples exhibited the highest average AMF spore density; about 106 spores' 100 g^{-1} dry soil (Fig. 4).

3.3.4 AMF population density in Dejen district

Average AMF spore density under the teff cropland differed significantly ($p < 0.05$) among the sampling locations in Dejen district. As shown in Fig. 5, average AMF spore density ranged between 44.33 spores 100 g^{-1} dry soil at sampling location one (S1) to 53.67 spores 100 g^{-1} at sampling location three (S3). In the contrary, the control samples exhibited considerably a higher average spore density of 123 spores 100 g^{-1} dry soil (Fig. 5).

3.4 Arbuscular mycorrhizal fungi spore densities at district level

We observed significant variations in average AMF spore density across the sampling districts. The highest average AMF spore density (56.67 spores 100 g^{-1} dry soil) was recorded in Debre Elias district under the wheat cropland, followed by 50.75 spores 100 g^{-1} dry soil in Aneded district under the teff cropland (Fig. 6).

Comparatively lower average spore densities were observed in Dejen and Awabel districts under teff cultivation/croplands (Fig. 6).

3.5 AMF population density in different crop types.

In the present study, average AMF spore density of varied significantly ($p < 0.05$) between crop types (wheat and teff), Wheat cropland/fields exhibited the highest average AMF spore density of 56.67 spores 100 g^{-1} dry soil and but teff cropland/ fields exhibited average AMF spore density (48.67 spores per 100 g) of soil (Fig. 7).

The comparative average spore density analysis between croplands and controls exhibited significant ($p < 0.05$) variation. Controls exhibited the highest average AMF spore density 100 g^{-1} dry soil compared to the croplands (Fig. 8).

3.6 AMF composition across sampling districts

In this study, a total of 38 AMF morphotypes/species belonging to three genera were identified (Table 3). Among these, the genus *Acaulospora* was the most diverse, comprising 26 morphotypes/species, followed by *Glomus* with 7 morphotypes and *Pacispora* with 5 morphotypes (Fig. 9).

3.7 AMF Genus isolation frequency in the Study Districts

The isolation frequency analysis indicated that the genus *Acaulospora* was the most prevalent, occurring in 96.7% of the samples with a relative abundance of 89.4% (Table 3). *Glomus* and *Pacispora* were recorded in 77.3% and 36.4% of the samples, with relative abundances of 21.3% and 17.01%, respectively (Table 3).

Table 3
Isolation Frequency, Relative Abundance, and Importance Value of AMF Genera across Sampling Districts

Genus name	Number of morphotypes	IF (%)	RA (%)	IV (%)	Status
Acaulospora	26	96.7%	89.4	93.1	Dominant
Glomus	7	77.3	21.3	49.3	Common
Pacispora	5	36.4	17.02	26.71	Common

This was followed by *Acaulospora myriocarpa*, with an isolation frequency of 77.3%, whereas *Glomus fulvus* occurred much less frequently, at 18.6%. A similar pattern was observed for relative abundance: *A. scrobiculata* was the most dominant species, comprising 26.1% of the total AMF community, followed by *A. myriocarpa* with 24.8%. The dominance of these species was further confirmed by their importance values (IV), with *A. scrobiculata* recording the highest IV (56.2%), followed by *A. myriocarpa* (51.1%). (Table 4).

Table 4
Isolation Frequency (IF), Relative Abundance (RA), and Importance Value (IV) of AMF Species across Different districts

S.N	AMF species	IF (%)	RA (%)	IV	Status
1	*Acaulosporalaevis*	9.09	4.35	6.72	Rare
2	*Glomusmelanosporum*	13.6	1.45	7.54	Rare
3	*Acaulosporascrobiculata*	86.4	26.1	56.2	Dominant
4	*Acaulosporamyriocarpa*	77.3	24.8	51.05	Dominant
5	*Acaulosporamellea*	4.55	1.45	3	Rare
6	*Glomusmagnicaule*	22.6	3.45	13.03	Common
7	*Acaulosporafoveata*	18.55	5.45	12	Common
8	*Acaulosporarehmii*	13.6	2.9	8.27	Rare
9	*Acaulospora lacunose*	4.55	1.45	3	Rare
10	*Acaulospora delicate*	19.09	4.52	11.81	Common
11	*Glomusfulvus*	18.6	5.45	9.82	Rare
12	*Glomusrubiformis*	13.6	1.45	7.54	Rare
13	*Glomusmulticaule*	9.09	1.45	5.27	Rare
14	*Acaulospora sp1*	13.6	1.45	7.54	Rare
15	*Pacisporafranciscana*	18.2	7.25	12.73	Common
16	*Glomus sp1*	9.09	1.45	5.27	Rare
17	*Acaulosporaspinose*	4.55	1.45	3	Rare
18	*Acaulospora sp2*	4.55	1.45	3	Rare
19	*Glomus sp2*	4.55	1.45	3	Rare
20	*Pacisporacorralloidea*	14.55	10.55	12.55	Common
21	*Acaulospora sp3*	4.55	1.45	3	Rare
22	*Acaulospora sp4*	4.55	1.45	3	Rare
23	*Acaulospora sp5*	4.55	1.45	3	Rare
24	*Acaulospora sp6*	9.09	1.45	5.27	Rare
25	*Acaulospora sp7*	4.55	1.45	3	Rare
26	*Acaulospora sp8*	13.6	1.45	7.54	Rare
27	*Glomus sp3*	9.09	1.45	5.27	Rare
28	*Glomus sp4*	4.55	1.45	3	Rare
29	*Glomus sp5*	4.55	1.45	3	Rare
30	*Acaulospora sp9*	4.55	1.45	3	Rare
31	*Pacisporadominikii*	14.55	11.45	13	Common

S.N	AMF species	IF (%)	RA (%)	IV	Status
32	*Acaulospora sp10*	4.55	1.45	3	Rare
33	*Acaulospora sp11*	4.55	1.45	3	Rare
34	*Acaulospora sp12*	4.55	1.45	3	Rare
35	*Acaulospora sp13*	4.55	1.45	3	Rare
36	*Acaulospora sp14*	9.09	1.45	5.27	Rare
37	*Acaulospora sp15*	9.09	1.45	5.27	Rare
38	*Pacispora sp1*	16.55	11.45	14	Common

3.8 Isolation Frequency, Relative Abundance, and Importance Values of AMF Species

In this study, variation was observed in the distribution and dominance of AMF species across the sampling districts and locations (Table 3). Among the identified species, *Acaulospora scrobiculata* exhibited the highest isolation frequency (86.4%), making it the most widespread species in the cropland soils examined.

3.9 AMF Genera status of recovery from the study districts

Data analysis of the AMF genera revealed distinct distribution patterns across the sampling districts. Genus *Acaulospora* was recovered from all the study districts (Table 5). All the three AMF genera, namely *Acaulospora, Glomus*, and *Pacispora*, were consistently recovered from Debre Elias and Dejen district croplands. However, *Glomus* and *Pacispora* were not recovered from the Awabel district and similarily, genus *Pacispora* was not recovered from Aneded district (Table 5).

Table 5
AMF Genera recovered from the study districts

Genera	Aneded	Awabel	Debre Elias	Dejen
Acaluspora	+	+	+	+
Glomus	+	-	+	+
Pacispora	-	-	+	+

3.10 AMF Genera recovered from wheat and teff croplands

In this study,both wheat and teff croplands exhibited equal genus richness, each harboring *Pacispora*, *Acaulospora*, and *Glomus (*Table 6*)*.

Table 6
AMF Genera recovered from wheat and teff croplands

Genera	Teff	Wheat
Acaluspora	+	+
Glomus	+	+
Pacispora	+	+

3.11 AMF Species Richness across Crop Types

When the frequency of occurrence was considered, *Acaulospora* was found to be the dominant genus in the both wheat and teff croplands, being recorded 14 and 12 times, respectively, whereas *Pacispora* was observed only 3 times in wheat cropland and 2 times under teff croplands .Among 38 AMF morphotypes, 21 species were recovered from wheat croplands, while 17 species were recovered from teff croplands (Table 7).

Table 7
AMF Genus Richness, Species Count per Genus, and Overall Species Richness (SR) Across Crop Types

Crop type	Genus richness	Genus name	Number of species of each Genus	Species richness(SR)
Wheat	3	*Acaluspora*	14	21
		Glomus	4	
		Pacispora	3	
Teff	3	*Glomus*	3	17
		Acaluspora	12	
		Pacispora	2	

4. DISCUSSION

AMF establish a mutual association with most land plants and help them survive in water and nutrient deficient conditions. They absorb and translocate plant nutrients [4], provide tolerance to the biotic and abiotic stresses conditions [16], they play significant role in improving soil structure, carbon sequestration and in the maintenance of resilient ecosystems [17].

It is well documented that a continuous and long term chemical fertilizer application to the croplands could reduce the plant and AMF symbiotic association. This is because if there is sufficient plant nutrients like P and N supply from the external sources, then plants depend on these external nutrient sources rather than forming ecologically important symbiotic association [2, 18].

In this study, there has been continuous and long term chemical fertilizer and chemical pesticide application in the past ten year (Table 1). The reduced average AMF spore density in all croplands compared to the relatively less disturbed controls could be attributed to these continuous and long term chemical N-P fertilizer application and excessive pesticide usage. Similarly, reduced average AMF spore densities from wheat and teff croplands were reported by Berza et al [9] and Getachew et al [19] from P and N fertilized croplands in northwestern and northern Ethiopia. The lower AMF population density could result from the reduced spore production which again attributed to the supply of less carbon from the host plants. But moderate phosphorus additions on fertile soils typically do not alter AMF community composition [20], prolonged N and P application can restructure AMF communities and decrease species diversity [1, 21].

In the present study, the wheat croplands exhibited higher average AMF spore density as well as greater genus and species richness compared to the teff croplands. The average AMF spore density recorded from wheat cropland was 56.67 spores 100 g^{-1} dry soil. Our result is in line with Berza et al [9] who have reported significantly higher average AMF spore from wheat rhizosphere in Debre Elias district compared to the teff rhizosphere from Enrata and Gozamin districts. However, our finding with respect to average AMF spore density is lower than those reported in previous studies. For example, Vyas et al. [22] reported AMF spore densities ranging from 66 to 154 spores per 50 g of dry soil in wheat rhizosphere, nearly double the densities observed in the current study. The difference could be attributed the fact that rhizosphere is rich in nutrients, metabolically active and supports much higher microbial population compared to the croplands.

Similarly, earlier studies from Central Ethiopia reported substantially higher AMF spore densities under different soil management and land-use conditions. For instance, Belay et al. [23] found average AMF spore densities ranging from 250

to 1110 spores 100 g^{-1} dry soil in low-input agricultural farmlands of Shewa Robit, central Ethiopia,. These differences are most likely due to variations in soil fertility, land-use history, crop management practices, and environmental conditions that regulate AMF abundance. In addition, farmers in the study area have been using pesticides for more than ten years (Table 1). The effect of pesticides on AMF spore density and community composition depends on multiple factors, including the chemical properties of the active ingredient, mode and dosage of application, soil type, and interactions with other soil microorganisms. Pesticides may have detrimental, neutral, or even beneficial effects, influencing spore germination, hyphae growth toward roots, colonization of root tissues, or internal fungal development [24]. Their impact can also be observed indirectly through changes in phosphorus uptake by mycorrhizal plants. Since AMF species differ in sensitivity, long-term pesticide use may alter species interactions and drive shifts in community structure, sometimes by modifying broader soil microbial populations. When AMF spore density and species diversity declines and reduced AMF colonization limits nutrient and water absorption, leading to lower crop growth and yields, soil microbial balance and ecosystem stability are weakened, making the soil more prone to degradation and nutrient loss and result in reduced soil fertility, poor plant performance, and greater dependence on chemical fertilizers [25].

Conclusion

The present study revealed that average AMF spore density varied significantly ($p < 0.05$) among sampling locations, districts, and crop types. The wheat croplands exhibited the highest average spore density 100 g^{-1} dry soil compared to the teff croplands. Among the sampling districts, Debre Elias with wheat cropland recorded the highest average spore density 100 g^{-1} dry soil compared to the other districts with teff croplands. *Acaulospora* was the dominant genus, being consistently recovered from all districts. Alternative soil fertility management practices such as crop rotation, organic soil amendments and reduced chemical fertilizer and pesticide application is recommended to enhance AMF spore density and species diversity in the croplands in the study areas.

Declarations

Declaration of conflict of interest

There is no competing interest to declare

Consent for publication

Not applicable

Ethics approval and consent to participate

This study has been reviewed and approved Ethical review committee of Department of Biology, Debre Markos University on December 2, 2023 by ref. number DMU/NCS/Biol/5/2/2023. An Informed consent was also obtained from all the farmland owners.

Funding source

declaration

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Author Contribution

KA, data collection, drat writing, revised manuscript; MP. Methods, AMF identification;JY, methods, data curation; FA, resources; LK, methods, resources; YD, methods, resources; DK, methods, data collection; SG; methods, Map; BB; conception; supervision, project management, revision of the manuscript, resources.

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Data Availability

Primary data can be provided on request

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Figures

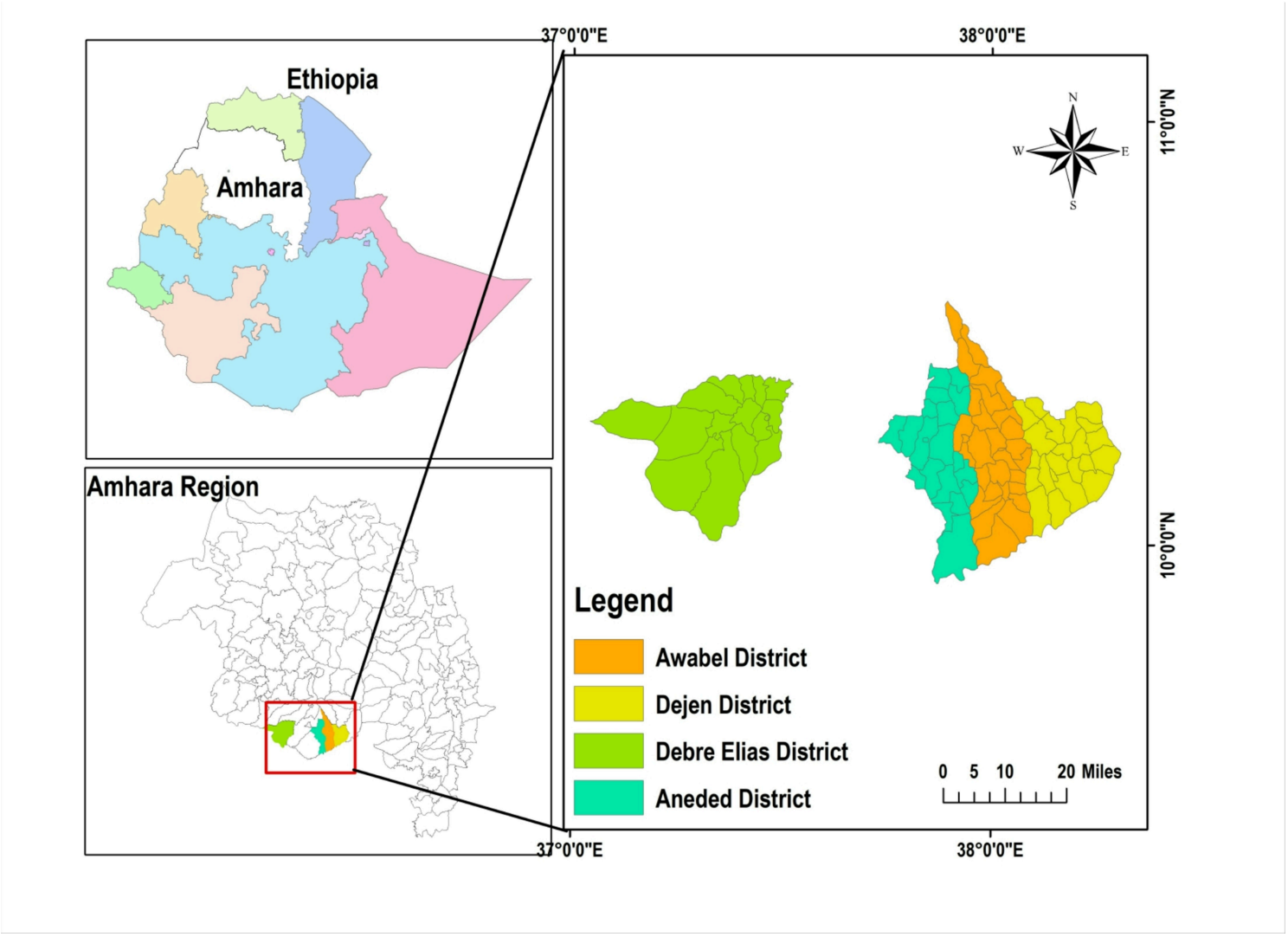


Figure 1

map of sampling districts

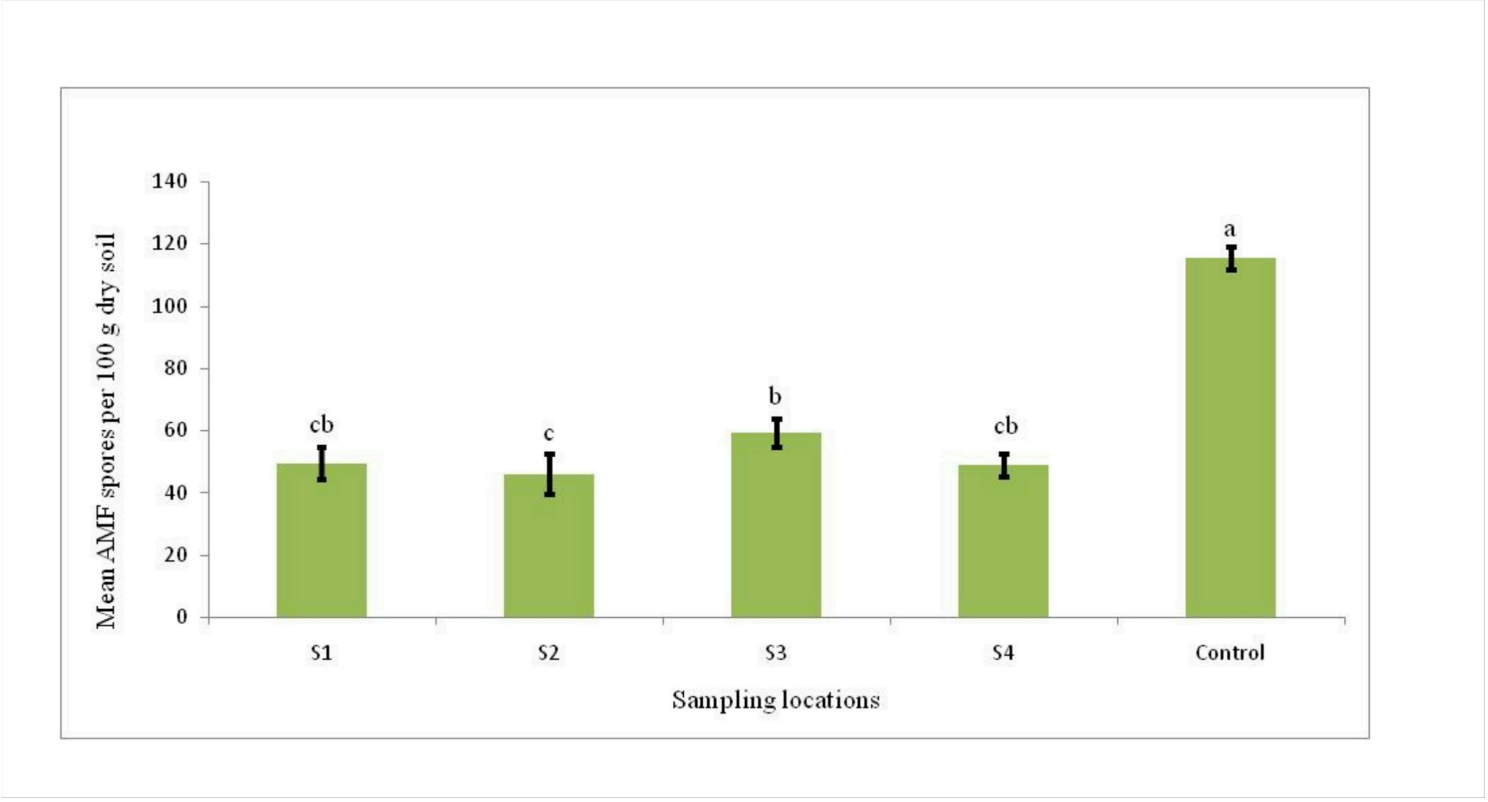


Figure 2

average AMF population density in Aneded district in teff croplands

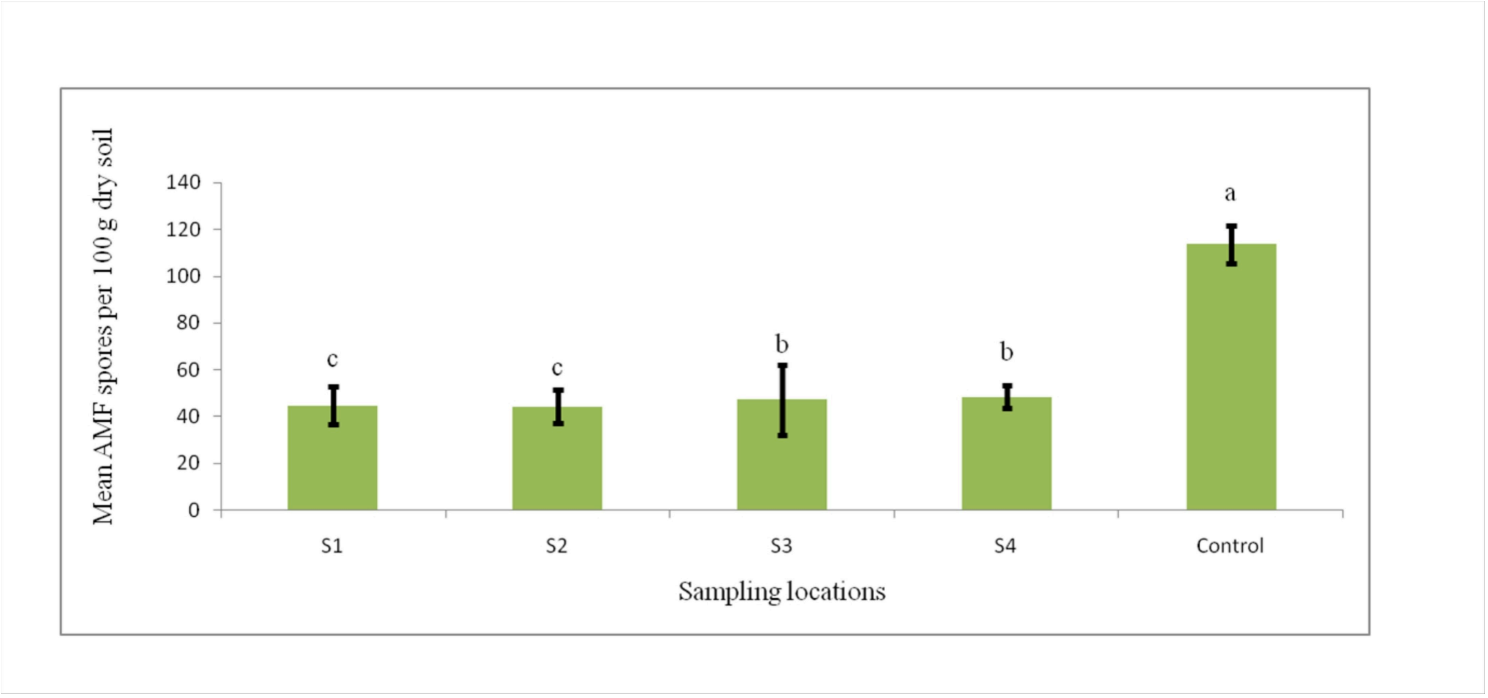


Figure 3

Average AMF population density at Awabel district from teff croplands

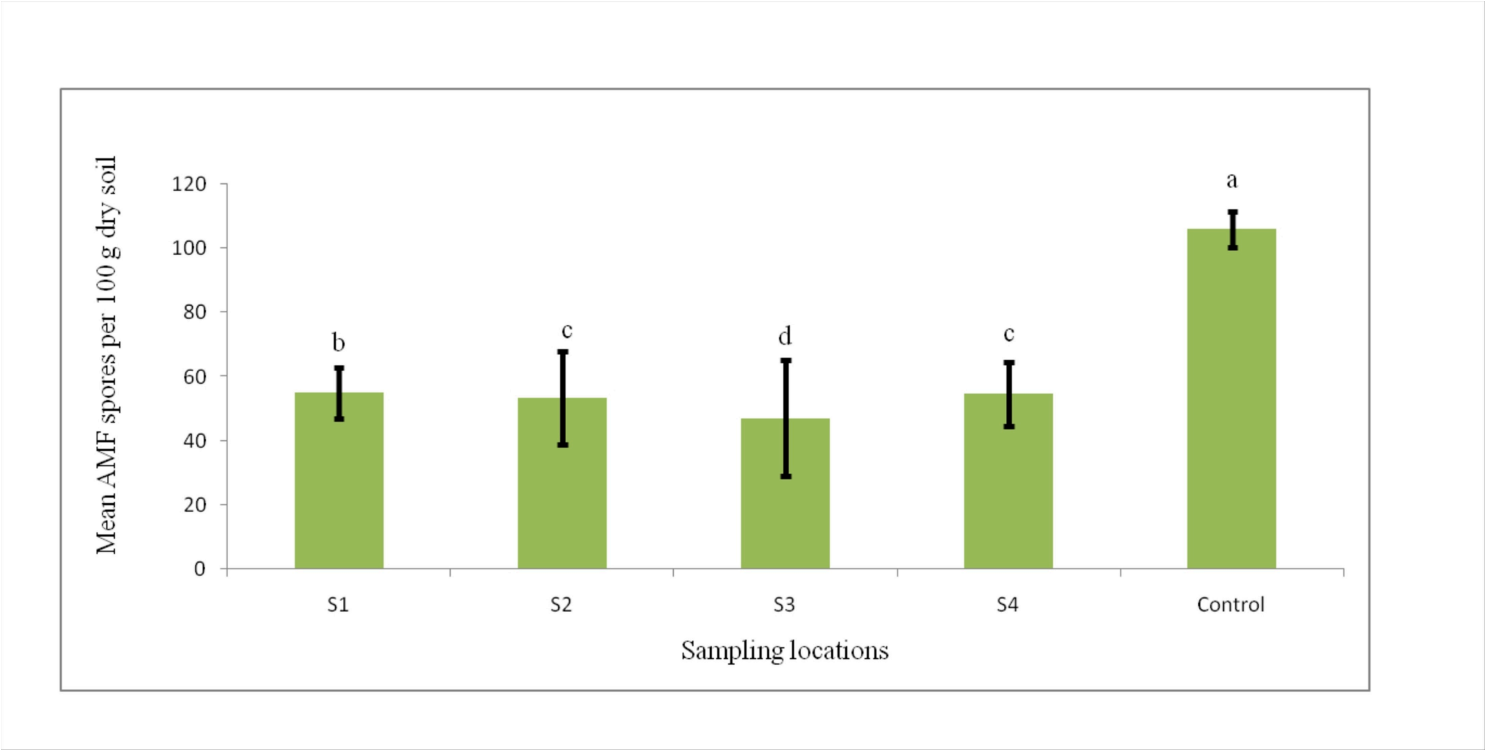


Figure 4

Average AMF population density in Debre Elias district from wheat croplands

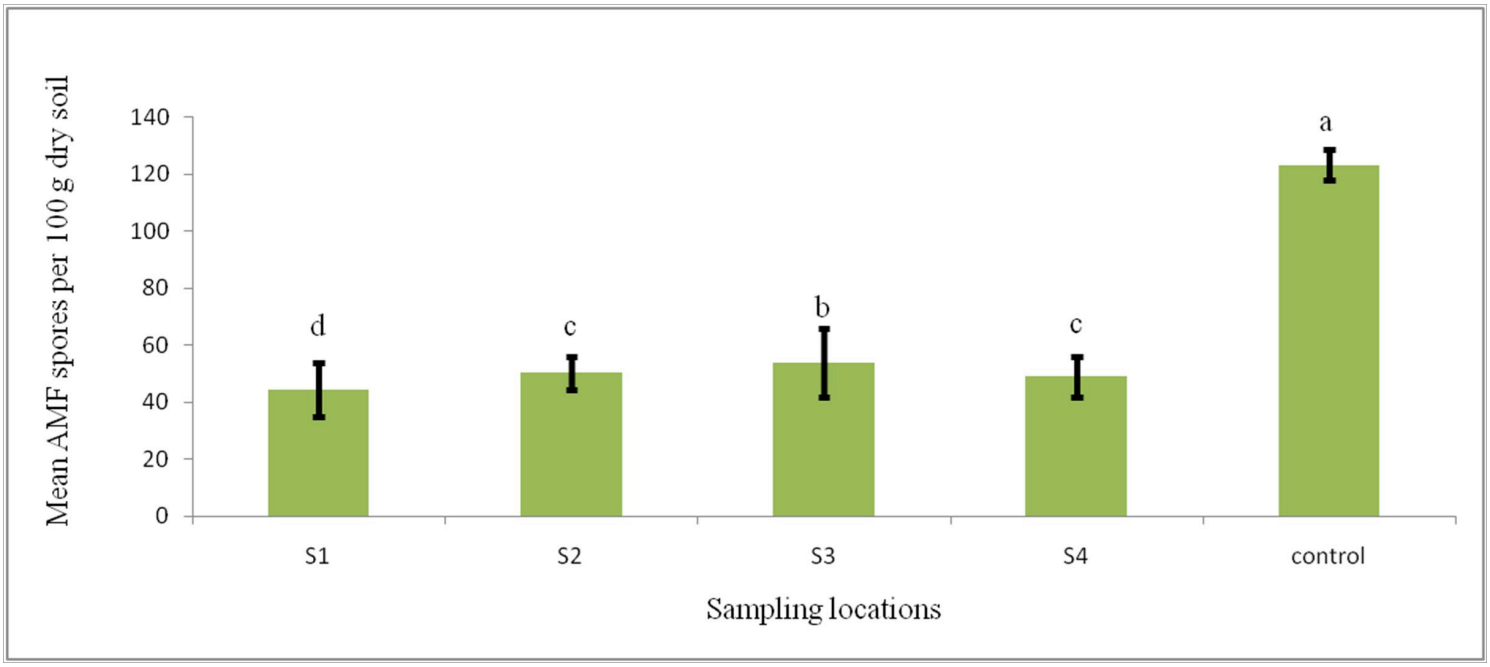


Figure 5

average AMF population density at Dejen district in teff croplands

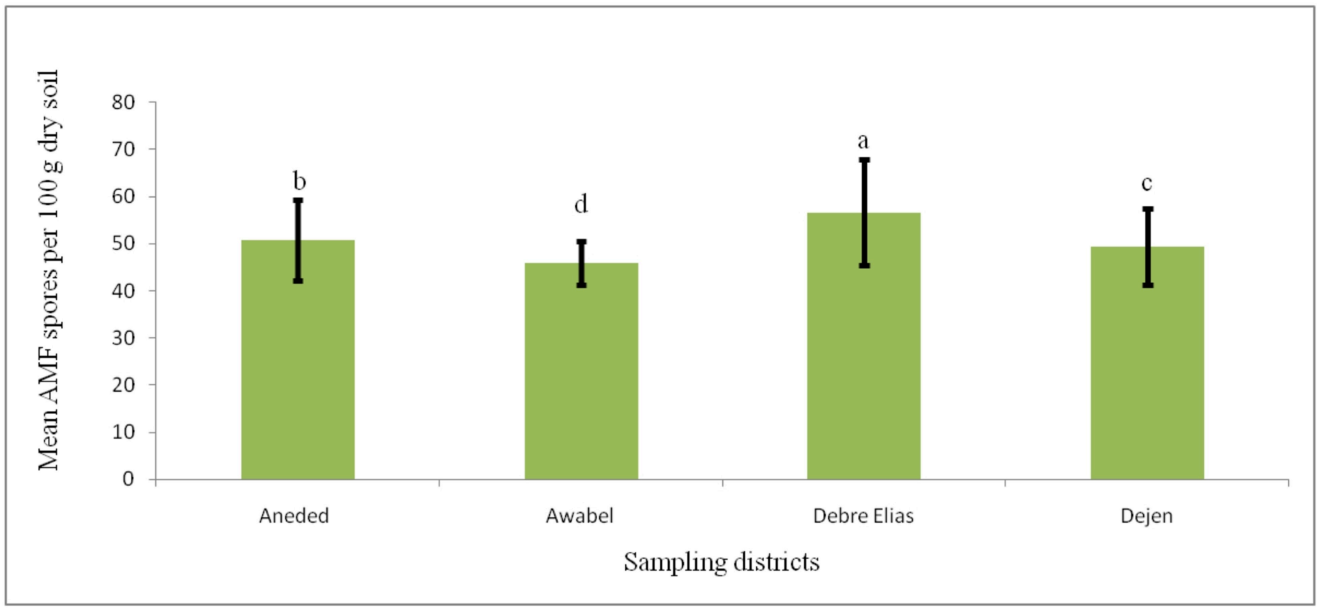


Figure 6

comparative average AMF spore density among sampling districts

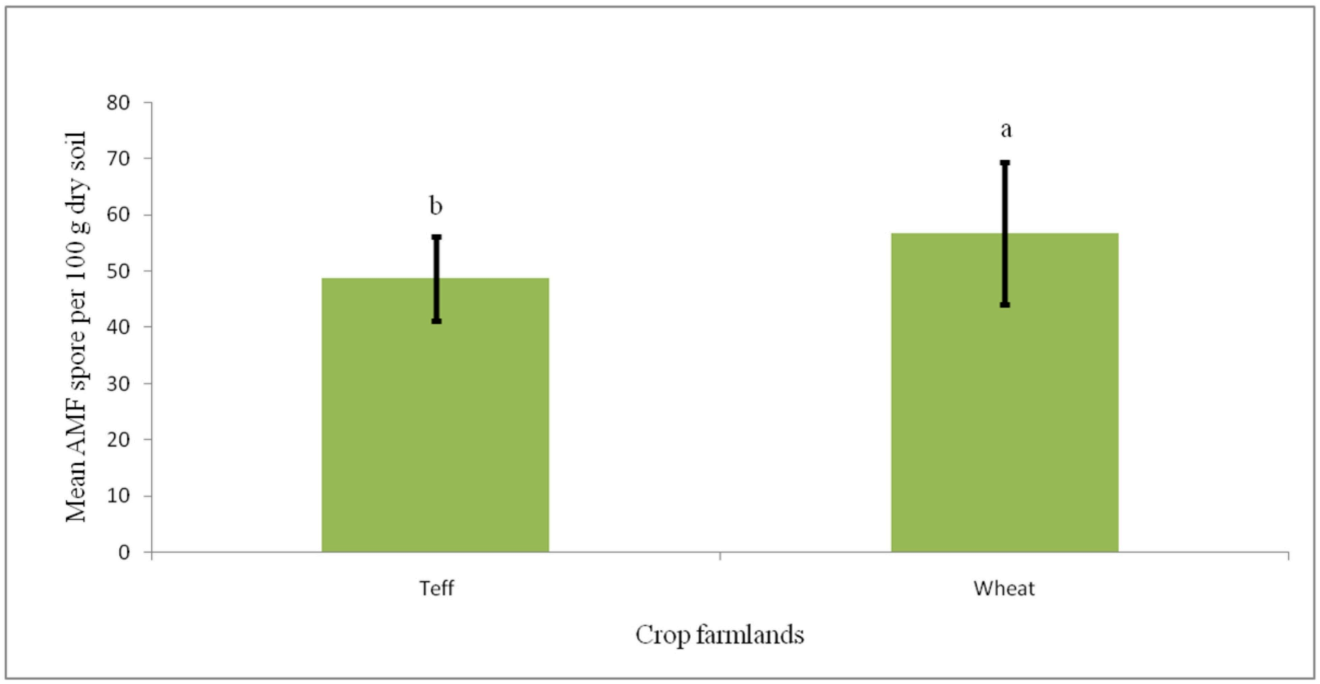


Figure 7

comparative average AMF spore density from teff and wheat croplands

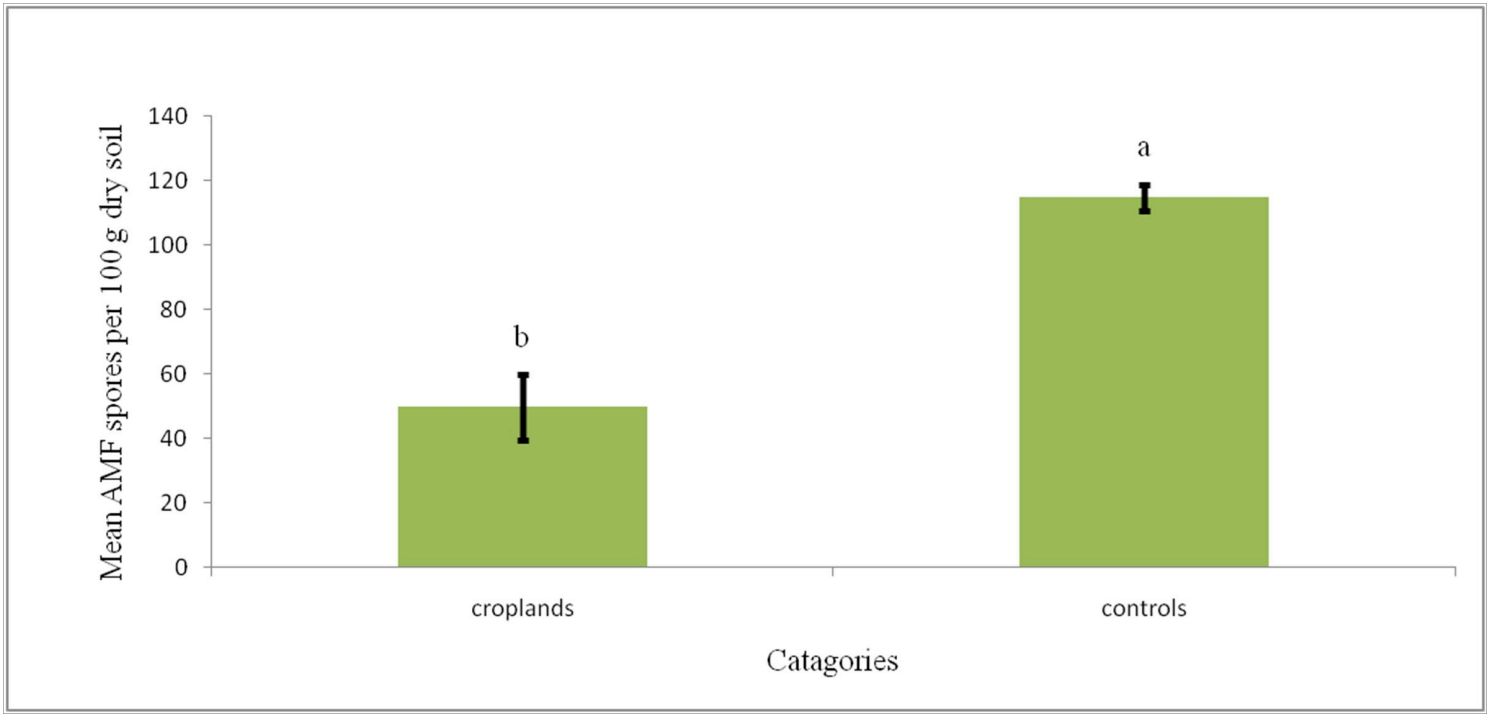


Figure 8

comparative average AMF spore density between croplands and controls.

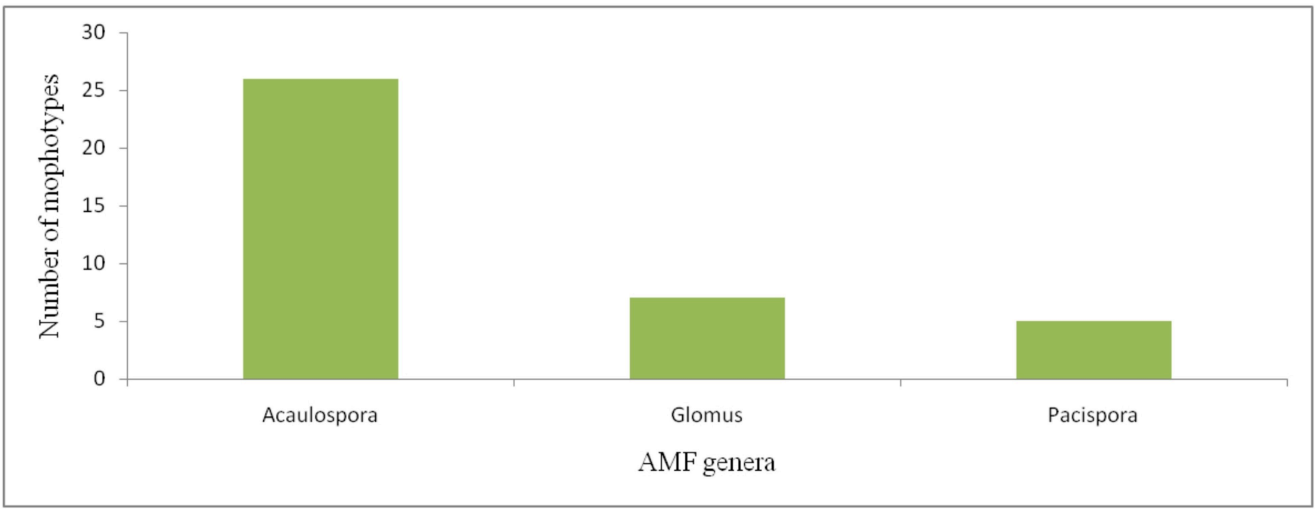


Figure 9

the number of AMF species/morphotypes among identified genera

Supplementary Files

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