Silvicultural Management Impact on Soil Mycorrhizal Association in Tigray Parklands Agroforestry Practices

Abstract

Arbuscular mycorrhizal fungi (AMF) play a vital role in plant growth and nutrient uptake. This study examined the effects of canopy distance and pruning frequency on arbuscular mycorrhizal fungi (AMF) spore density and root colonization in tree species of the Hawzen District, Northern Ethiopia. Experimental plots were established with varying distances from the canopy and pruning regimes. AMF spore density ranged from 63.33 to 277.67 spores per 100 g of dry soil, with a mean of 147.25 spores. Despite different canopy distances and pruning frequencies, no significant variations in spore density were observed, suggesting an uneven distribution. All essential AMF structures were found in the tree roots, including hyphae, vesicles, and arbuscules. Total root colonization (TRC) was 95.36%, with arbuscular colonization (AC) at 13.10%, vesicular colonization (VC) at 9.11%, hyphal colonization (HC) at 31.63%, and mycorrhizal hyphal colonization (MHC) at 20.68%. Statistical analysis revealed significant differences in TRC, AC, HC, and MHC among treatments, indicating a robust mycorrhizal association. However, VC remained unaffected. Canopy distance and pruning frequency have influence AMF distribution, The presence of all AMF structures in the tree roots emphasizes the crucial role of mycorrhizal symbiosis in promoting plant growth and resilience in the Hawzen District.

Key words: Arbuscular mycorrhizal, pruning frequencies, canopy distances

Introduction

Canopy management, specifically tree training and pruning, significantly influences a tree's ability to capture sunlight. Optimal tree architecture achieved through careful training, maximizes light interception and subsequent productivity (Bhat *et al*., 2021). Canopy structure, characterized by leaf arrangement and

distribution, directly impacts microclimate and photosynthesis (Caldwell *et al*., 1986). Leaf area index (LAI), a measure of leaf area per ground surface area (Chen and Black, 1992, Bréda, 2003), is a crucial determinant of light interception efficiency. Coupled with stand structure tree number, spacing, and size distribution canopy architecture plays a pivotal role in ecological and climate models. Understanding these relationships is essential for optimizing tree growth and productivity (Peng, 2000).

Farmers' management of *Faidherbia albida* trees is significantly influenced by the perceived value of their products. Prior to a severe drought in Burkina Faso, the primary motivation for pruning *F. albida* was to mitigate their environmental impact. However, as the trees' economic importance has risen, management practices have evolved to prioritize fruit production (Tscharntke *et al*., 2011). Pruning has been shown to increase leaf biomass, reduce canopy size, and stimulate regrowth (Sillett *et al*., 2018). Regular pruning can even induce rejuvenation in older trees. While these changes can be beneficial, it's important to note that pruning drastically reduces pod production. Optimal pruning practices, therefore, must balance the trade-offs between increased leaf biomass and fruit yield (Bevacqua *et al*., 2021).

Parkland agroforestry systems are known to enhance soil properties compared to open areas. Increased organic matter accumulation under tree canopies improves soil microbial activity, decomposition rates, and physical structure (Rhoades, 1996). *F. albida's* unique phenological characteristics involves shedding leaves during the summer and growing leaves during the dry season (Kariba, 2013). Previous studies have documented significant increases in soil microbial biomass, reduced soil bulk density, and enhanced water infiltration rates under tree canopies (Abdella *et al*., 2020, Abdella and Nigatu, 2021, Clermont-Dauphin *et al*., 2023). Additionally, increased macrofauna activity, particularly termites, has been linked to improved soil structure. These findings highlight the critical role of parkland trees in improving soil health and ecological balance (Marsden *et al*., 2020, Duran-Bautista *et al*., 2023).

Faidherbia albida is a widespread African tree species renowned for its ecological adaptability (Tegegne, 2008). Its unique growth pattern, with leaves retained during the dry season and shed during the rainy season, has made it a cornerstone of traditional agroforestry systems. This inverse phenology minimizes competition with crops and provides crucial fodder during lean periods (Soni *et al*., 2017). Furthermore, the species exhibits significant genetic and morphological variation, highlighting its potential for diverse applications.

The influence of canopy management on *Faidherbia albida* and its subsequent impact on soil-plant-mycorrhiza interactions remains poorly understood. While the importance of this species in parkland agroforestry systems is well-established, specific research on the effects of tree size and pruning frequency on arbuscular mycorrhizal fungi (AMF) associations is limited. Previous studies (Sileshi, 2016, Dilla *et al*.,

2019, Sida *et al*., 2018) have shown that *F. albida* canopies enhance understory productivity. However, the mechanisms underlying these benefits, particularly the role of AMF in nutrient cycling and soil structure, are not fully elucidated. This study aims to fill this knowledge gap by investigating the relationship between tree size, pruning practices, and AMF colonization in *F. albida* trees.

Materials and Methods

Area description

The field experiments were conducted in Hawzen District, located in the Northern Ethiopian highlands. Situated at 13° 58' 39" N latitude and 39° 25' 45" E longitude, Hawzen has an altitude of 2263 meters above sea level. This region falls within the tepid to cool sub-moist mountains plateau agro-ecological zone.

Known for its degraded soils, particularly low in organic matter, Hawzen District represents a challenging environment for agriculture. The annual mean maximum temperature in this area is 24°C, while the mean minimum temperature is 7.7°C.

According to traditional agroecological classification, 60% of Hawzen falls within the midlands (1500-2500 meters above sea level), 35% in the lowlands (500-1500 meters), and 5% in the highlands (2500-3500 meters). The district experiences a weakly bimodal rainfall pattern, with smaller showers occurring from March to May and a longer rainy season during June to August. Annual rainfall in Hawzen ranges from 470 to 613 millimeters (Kahsay *et al*., 2019).

METHOD

Study plant selection

Before conducting the experiment, isolated *F. albida* trees grown on cultivated lands will be selected from farmer's fields of parkland agroforestry systems, after doing reconnaissance survey. *F. albida* commonly intercropped with most agricultural crops (Weil and Mughogho, 1993). *F. albida* trees selected for the study will in the same soil type with the differential canopy size (full canopy size and trees not researched full canopy size) by considering the age of the trees to reach full canopy size and pruning frequency based on the number of pruning of branches of *F. albida* trees (i.e. 1-5 times, 6-10 times, 11-15 times, >15 times, and trees not yet pruned considered as the same age and in the same soil type with the other pruned trees) by asking the local residents of the study site.

Experimental design and field layout

To conduct the experiment, a field experiment will be conducted during the cropping season (from May to September). *F. albida* trees grown on farmer's wheat fields in a parkland agroforestry system will be selected based on their canopy size and pruning frequency. Hence, eight treatments will be laid out in a completely randomized Design (CRD) with six replications (Table 1).

Data collection

Soil sampling

Prior to soil sampling, trees grown at farmers cultivated fields will be selected based on their canopy size (full canopy size and not reached full canopy size) and pruning frequency (1-5 times, 6-10 times, 11-15 times, and trees not yet pruned considered as the same age and in the same soil type with the other pruned trees). Soil samples will be collected for spore analysis and assessment of AM fungal colonization. Surface soil (approximately 1–2 cm) will be removed, and soil cores of 0-30 cm depth will be collected including fine roots and rhizosphere soils of the host plant using a cylindrical soil corer of 10cm internal diameter (Hailemariam *et al*., 2013). Soil samples were also collected away the canopy of the selected *F. albida* trees. Each tree species was replicated three times for sampling of soil and roots. Four rooting-zone soil samples with fine roots was collected in four directions perpendicular to each other from the selected plant species, and the four soil samples per individual tree from each land use was mixed thoroughly to form a composite soil sample (Hailemariam *et al*., 2013).

Generally, from each selected isolated individual trees one composite soil sample (approximately 400 g), and a total of 42 composite soil samples will be taken for extraction of AM fungal spores. The soil samples for the assessment of mycorrhizal colonization, live fine roots of *F. albida* trees will be collected, chopped down into to 1cm long segments and placed in 50% ethanol and stored at room temperature until clearing and staining (Brundrett *et al*., 1996).

Assessment of AMF Spores

Spores will be extracted from 100g of air-dried sub-samples representing each horizontal distance from tree trunk by wet sieving and decanting method followed by flotation-centrifugation in 50% sucrose (Brundrett *et al*., 1996). The air-dried sub-samples of soil will be suspended in water for 30 seconds and decanted over a series of sieves with 750, 250, 100 and 40 μm mesh size. Soil material will be recovered from each sieve, suspended in water, and centrifuge at 2000 revolution per minutes (RPM) for 5 minutes (Brundrett *et al*., 1996). After decanting the supernatant, each soil-spore mixture of the pellets will be re-suspended in sucrose solution (50%) and centrifuged for 1 min at 2000 RPM. The supernatant containing spores is filtered

under vacuum on filter paper and then transferred into round Petridis that has gridline marked at the bottom to form 1cm square and finally counted using a stereoscope Microscope (Hailemariam *et al*., 2013).

Assessment of AM colonization

Live fine roots collected from the rhizosphere soil of *F. albida* trees for the assessment of mycorrhizal colonization will be chopped down into to 1cm long segments and placed in 50 % ethanol and stored at room temperature, and then cleaned with 10 % KOH and autoclaved at 121 ^{0}C for 15 min. cleared root samples will then be acidified with 3% HCl (v/v) for 8-9 minutes. Finally, they will be transferred into a staining solution of Trypan blue (0.05% w/v) in lactoglycerol, and autoclaved at 121^{0}C for 15 minutes (Brundrett *et al*., 1996). Afterwards stained roots were left in a de-staining solution (50% glycerol) to remove colorations from empty root cells. Finally, from each representing sample nine randomly selected stained roots will be prepared and examined at 100–400× magnification under a microscope for the presence of AM fungal structures. Root colonization of individual species was estimated by the gridline intersect method as described by (Giovannetti and Mosse, 1980).

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) to assess treatment effects. The AM fungi associations was subjected to analysis of variance using R software. Significant differences between means were determined by LSD at 5% probability level. Differences on AM fungi associations was subjected to analysis of variance using SAS statistical software (SAS, version 9). When the analysis of variance (ANOVA) shows significant differences (at $P<0.05$), a mean separation was made using Duncan's multiple range test.

Result and Discussion

Variation of AMF spore density within canopy distance and pruning frequency

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the roots of most terrestrial plants, enhancing nutrient uptake, particularly phosphorus, and improving plant resilience to environmental stressors (Smith and Read, 2010). The distribution of AMF spores in soil can be influenced by various factors, including canopy cover, soil type, and management practices such as pruning (Bever *et al*., 2001, Widiastuti and Topani, 2023). This study aims to assess the variation in AMF spore density concerning canopy distance and pruning frequency, contributing to our understanding of AMF ecology in managed ecosystems.

The results indicate that AMF spore density ranged from 63.33 to 277.67 spores per 100 g of dry soil, with an overall mean of 147.25 spores per 100 g of dry soil (Table 1). This finding was similar to Birhane *et al*. (2017). The analysis revealed no significant differences ($P < 0.05$) in spore density among the various pruning frequencies and canopy distances, suggesting that the distribution of AMF spores is relatively uniform across these variables (Table 2, 3, 4).

The lack of significant variation in AMF spore density across different pruning frequencies and canopy distances may reflect the resilience of AMF populations to changes in environmental conditions or management practices. The studies have shown that AMF spore can maintain stable populations despite fluctuations in host plant availability and soil conditions (Helgason *et al*., 1998). This finding underscores the importance of considering AMF dynamics in ecosystem management, particularly in agroforestry and horticultural systems.

Spore Density in Relation to Pruning Frequency

The spore density highest in 11-15 pruning frequency which is 169.82 spores per 100 g and lower in highest pruning frequency which is 91.2 spores per 100 g, but have no significance difference between the treatment (Table 3). The results indicated that spore density peaked at a pruning frequency of 11-15, with a mean value of 169.82 spores per 100 g. In contrast, the highest pruning frequency recorded a mean spore density of 91.2 spores per 100 g. Despite these observed differences, statistical analysis revealed no significant differences between the treatment groups ($p > 0.05$), indicating that the variations in spore density may not be attributable to the pruning frequency alone.

The findings suggest that while spore density is influenced by pruning frequency, the lack of statistical significance implies that other environmental or biological factors may also play a critical role in determining spore density (Sivakumar, 2013). Factors such as humidity, temperature, and the presence of competing flora could potentially affect spore germination and growth, warranting further investigation. The highest spore density at the 11-15 pruning frequency may indicate an optimal balance between plant health and spore proliferation (Zheng *et al*., 2024), but the absence of significant differences suggests that the relationship is complex and multifactorial. Future research should aim to explore these interactions in greater depth, potentially incorporating additional variables such as soil health, plant variety, and climatic conditions.

Spore Density Distribution within Tree Canopies

results demonstrated that spore density was higher significantly within the canopy, with a mean value of 147.91 spores per 100 g, compared to a lower mean of 95.5 spores per 100 g at the base of the tree (Table

3). This is may be due to warmer temperatures at the canopy level can promote spore growth and development (Dewi *et al*., 2021), and higher humidity levels in the canopy can create a favorable environment for spore survival and dispersal (Boateng and Lewis, 2015). However, statistical analysis revealed no significant differences between the treatment groups ($p > 0.05$), indicating that the observed variations may not be solely attributable to vertical positioning.

The findings suggest that while spore density is higher within the canopy, the lack of statistical significance implies that other environmental factors may influence spore distribution. Factors such as light availability, humidity, and airflow within the canopy could affect spore germination and growth (Cardoso *et al*., 2003), necessitating further investigation.

The higher spore density within the canopy may reflect a more favorable microenvironment for fungal growth, but the absence of significant differences indicates that the relationship is complex. Future research should explore additional variables, including tree species, canopy structure, and seasonal variations, to gain a more comprehensive understanding of spore dynamics.

Variation of Mycorrhizal association in relation to both pruning frequency and canopy distance

All arbuscular mycorrhizal fungi (AMF) structures were observed in the root samples. The mean total root colonization (TRC) was 95.36, with arbuscular colonization (AC) at 13.10, vesicular colonization (VC) at 9.11, hyphal colonization (HC) at 31.63, and mycorrhizal hyphal colonization (MHC) at 20.68. Statistical analysis revealed significant differences in TRC, AC, HC, and MHC among the treatment groups ($p < 0.05$), suggesting a robust mycorrhizal association within the sampled tree species. However, no significant differences were found in vesicular colonization (VC).

The predominance of hyphal colonization (HC) suggests that these trees have established a strong mycorrhizal relationship, which is essential for nutrient acquisition, particularly in nutrient-poor soils. The findings align with those of (Moreira-Souza *et al*., 2003), who reported similar percentages of AMF structures in various tree species. The presence of arbuscules indicates active nutrient exchange, while vesicles may serve as nutrient storage for the fungal symbionts.

The results demonstrated that the highest total root colonization (TRC) was significantly higher at the highest pruning frequency and within the canopy, with a mean value of 133. In contrast, the lowest TRC was observed at the 11-15 pruning frequency and far from the canopy, measuring 60. This is due to the canopy might have a higher concentration of organic matter from fallen leaves and twigs, which can decompose and release nutrients into the soil, therefore these nutrients can promote root growth and

colonization (Ma *et al*., 2022), and Frequent pruning can stimulate the plant to produce more roots in response to the stress of losing branches. This increased root growth could lead to greater colonization by beneficial microorganisms (Khan *et al*., 2021). These nutrients can promote root growth and colonization The arbuscular colonization (AC) was highest at the highest pruning frequency and within the canopy, with a mean of 27.3, while the lowest AC was recorded under no pruning conditions and far from the canopy, at 3.6. Vesicular colonization (VC) followed a similar trend, with the highest value of 15.75 at the highest pruning frequency and within the canopy, and the lowest value of 2.14 under no pruning conditions and far from the canopy. Mycorrhizal hyphal colonization (MHC) also exhibited significant differences, with a peak of 36.06 at the highest pruning frequency and within the canopy, compared to a low of 5.4 at no pruning and far from the canopy.

The results indicate a clear relationship between pruning frequency, canopy position, and mycorrhizal colonization. The significantly higher TRC, AC, VC, and MHC at the highest pruning frequency and within the canopy suggest that these conditions may create a more favorable environment for mycorrhizal fungi. Factors such as increased light availability, improved air circulation, and reduced competition may contribute to enhanced fungal colonization. Conversely, the low colonization rates observed under no pruning conditions and far from the canopy highlight the potential negative impact of reduced management practices on mycorrhizal associations. These findings underscore the importance of proper pruning techniques in promoting healthy root systems and enhancing plant growth through improved mycorrhizal colonization.

Mycorrhizal association in Relation to Pruning Frequency

Our findings indicate that the frequency of pruning significantly influenced the colonization of arbuscular mycorrhizal fungi (AMF) in the roots of *F. albida*. Statistical analysis revealed significant differences in TRC, AC, VC, HC, and MHC among the treatment groups ($p < 0.05$). The highest total root colonization (TRC) and arbuscular colonization (AC) were observed at the highest pruning frequency with a mean of 108.56 and 20.63, respectively. However, vesicular colonization (VC), hyphal colonization (HC), and mycorrhizal hyphal colonization (MHC) were highest at 6-10 pruning frequencies, reaching values of 12.03, 63.10, and 29.14, respectively. Conversely, the lowest levels of TRC, AC, HC, and MHC were recorded in the absence of pruning with a mean of 91.78, 6.46, 11.75, and 11.91, respectively (Table 6). Our findings also contribute to the understanding of the specific AMF structures affected by pruning. While AC was highest at the most frequent pruning interval, VC, HC, and MHC were optimal at intermediate

frequencies. This suggests that different AMF structures may respond differently to pruning, potentially due to their distinct ecological roles.

These results align with previous studies that have explored the relationship between pruning practices and AMF colonization. For example, (Holland *et al*., 2019). These studies generally suggest that moderate levels of pruning can enhance AMF colonization, potentially due to increased root growth and nutrient availability. However, excessive pruning may disrupt the delicate balance between the host plant and AMF, leading to reduced colonization.

Mycorrhizal association in Relation to Canopy Distance

Our findings indicate a significant spatial variation in mycorrhizal colonization within the study area. Statistical analysis revealed significant differences in TRC, AC, HC, and MHC among the treatment groups ($p < 0.05$), suggesting a robust mycorrhizal association within the sampled tree species. However, no significant differences were found in vesicular colonization (VC).

Roots sampled from under the canopy exhibited significantly higher levels of total root colonization (TRC), arbuscular colonization (AC), vesicular colonization (VC), hyphal colonization (HC), and mycorrhizal hyphal colonization (MHC) compared to those collected from areas distant from the canopy. Specifically, TRC, AC, VC, HC, and MHC were 107.93, 16.5, 10.89, 34.67, and 24.98 higher under the canopy, respectively, compared to 86, 9.41, 6.77, 26.22, and 15.64 in areas far from the canopy (Table 7).

These results align with previous studies that have demonstrated a positive correlation between canopy cover and mycorrhizal colonization (Birhane *et al*., 2018). The increased light intensity and nutrient availability under the canopy likely create a more favorable environment for mycorrhizal fungi to thrive. The higher levels of mycorrhizal colonization observed in this study may contribute to enhanced plant growth and nutrient uptake in the canopy regions. However, it's important to note that other factors, such as soil properties, plant species, and environmental conditions, can also influence mycorrhizal colonization (Soudzilovskaia *et al*., 2015).

Conclusion and recommendation

Conclusions

This research demonstrates that the distribution of arbuscular mycorrhizal fungi (AMF) spores is influenced by canopy distance and pruning frequency, but no significant differences were found among treatments of

spore density. Future studies should investigate the factors driving these distribution patterns and their impact on plant health and soil fertility.

Our analysis of tree roots revealed the presence of all essential AMF structures, with hyphal colonization being particularly prominent, and significance difference on AMF. These findings emphasize the crucial role of mycorrhizal symbiosis in promoting plant growth and nutrient acquisition, aligning with previous research.

Recommendations

To advance our understanding of arbuscular mycorrhizal fungi (AMF) and their ecological roles, future research should focus on: (1) investigating the factors that influence AMF distribution, including environmental conditions, plant species, and interactions with other microorganisms; (2) conducting long-term monitoring of AMF colonization to assess temporal changes and impacts on plant health; (3) exploring the potential applications of AMF inoculation in agricultural systems to enhance crop yields and sustainability; and (4) developing policies and practices that protect and promote AMF diversity and function in natural ecosystems, contributing to sustainable land management.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist

Table 1. Experimental treatments of the study area

SN	Pruning frequency	Canopy Distance
1	Not pruning of tree	With in canopy (1 – 3 m radius)
2	Pruning frequency of trees 1-5 times;	Near to canopy (4 – 6 m radius)
3	Pruning frequency of trees 6-10 times	Far from canopy (10 – 12 m radius)
4	Pruning frequency of tree 11-15 times	
5	Pruning frequency of trees > 15 times	

Table 2. The impact of pruning frequency and canopy distance on spore abundance

Pruning Frequency	Canopy Distance	Total spore density
No Pruning	Base of tree	132.33 (±59.5)
	Within canopy	172.00 (±40.2)
	Near to canopy	113.67 (±50.3)
	Far from canopy	123.00 (±46.5)
1 - 5 Pruning	Base of tree	90.33 (±42.0)
	Within canopy	111.33 (±42.6)
	Near to canopy	197.00 (±101.3)
	Far from canopy	214.00 (±52.0)
6 - 10 Pruning	Base of tree	150.00 (±78.5)
	Within canopy	192.67 (±92.6)
	Near to canopy	118.00 (±50.0)
	Far from canopy	112.33 (±29.9)
11 - 15 Pruning	Base of tree	131.33 (±35.5)
	Within canopy	277.67 (±48.4)
	Near to canopy	172.67 (±16.4)
	Far from canopy	166.33 (±52.9)
>15 Pruning	Base of tree	98.67 (±30.5)
	Within canopy	163.33 (±40.9)
	Near to canopy	145.00 (±98.6)
	Far from canopy	63.33 (±22.0)
Mean		147.25
CV		10.59
F value		0.7584
P value		0.7378

Post-hoc multiple comparison of pruning frequency and canopy distance using two-way ANOVA ($\alpha = 0.05$)

Table 3. The impact of pruning frequency on spore abundance

Pruning Frequency	Total spore density
>15 Pruning	91.20 (±1.25)
1 - 5 Pruning	120.23 (±1.24)
11 - 15 Pruning	169.82 (±1.13)
6 - 10 Pruning	97.72 (±1.36)
No Pruning	107.15 (±1.27)
Mean	117.23
CV	16.60528
F value	1.208
Pr(>F)	0.3179

Post-hoc multiple comparison of pruning frequency using one way ANOVA ($\alpha = 0.05$)

Table 4. The impact of canopy distance on spore abundance

Canopy Distance	Total spore density
Base of tree	95.50 (±1.21)
Far from canopy	112.20 (±1.20)
Near to canopy	107.15 (±1.28)
Within canopy	147.91 (±1.22)
Mean	2.06
CV	16.81
F value	0.804
Pr(>F)	0.4969

Post-hoc multiple comparison of canopy distance using one way ANOVA ($\alpha = 0.05$)

Table 5. The impact of pruning frequency and canopy distance on root colonization

Pruning Frequency	Canopy Distance	Total root colonization (TRC)	Arbuscular colonization (AC)	Vesicular colonization (VC)	Hyphal colonization (HC)	Mycorrhizal Hyphal colonization (MHC)
No Pruning	Within canopy	100.67 (±6.6)^{abc}	9.94 (±3.8)^{ab}	10.04 (±6.5)	18.62 (±2.1)^{ab}	17.52 (±8.6)^{ab}
	Near to canopy	85.67 (±4.1)^{abc}	5.86 (±1.2)^{a}	6.60 (±4.9)	18.62 (±2.1)^{ab}	12.81 (±5.8)^{ab}
	Far from canopy	89.00 (±5.0)^{abc}	3.60 (±1.4)^{a}	2.14 (±1.0)	4.68 (±1.5)^{a}	5.40 (±2.0)^{a}
1 - 5 Pruning	Within canopy	93.67 (±3.9)^{abc}	7.72 (±1.7)^{a}	10.59 (±3.6)	24.55 (±1.5)^{ab}	18.96 (±4.5)^{ab}
	Near to canopy	115.67 (±14.2)^{bc}	13.50 (±4.0)^{ab}	10.8 (±3.1)	28.84 (±1.9)^{ab}	23.65 (±7.7)^{ab}
	Far from canopy	95.67 (±7.7)^{abc}	9.95 (±3.1)^{ab}	7.38 (±2.1)	19.05 (±1.9)^{ab}	16.93 (±5.8)^{ab}
6 - 10 Pruning	Within canopy	103.0 (±15.0)^{abc}	16.36 (±5.1)^{ab}	6.56 (±0.9)	31.62 (±1.7)^{ab}	21.21 (±5.3)^{ab}
	Near to canopy	96.00 (±5.8)^{abc}	11.97 (±4.4)^{ab}	7.16 (±1.1)	20.42 (±2.3)^{ab}	15.95 (±6.0)^{ab}
	Far from canopy	94.33 (±6.9)^{abc}	3.84 (±0.8)^{a}	4.18 (±1.6)	7.59 (±1.1)^{ab}	7.40 (±0.9)^{a}
11 - 15 Pruning	Within canopy	109.33 (±8.8)^{abc}	20.94 (±1.1)^{ab}	11.51 (±4.2)	69.18 (±1.4)^{b}	31.13 (±2.7)^{ab}
	Near to canopy	61.67 (±16.7)^{ab}	15.59 (±1.6)^{ab}	13.29 (±2.6)	60.26 (±1.1)^{ab}	28.87 (±7.1)^{ab}
	Far from canopy	60.00 (±20.0)^{a}	15.29 (±4.5)^{ab}	11.31 (±2.1)	58.88 (±1.3)^{ab}	27.41 (±3.4)^{ab}
>15 Pruning	Within canopy	133.00 (±14.5)^{c}	27.53 (±4.6)^{b}	15.75 (±1.8)	51.29 (±1.4)^{ab}	36.06 (±2.9)^{b}
	Near to canopy	101.67 (±1.5)^{abc}	19.98 (±4.7)^{ab}	10.51 (±0.9)	33.88 (±1.6)^{ab}	25.87 (±4.7)^{ab}
	Far from canopy	91.00 (±4.5)^{abc}	14.38 (±3.6)^{ab}	8.85 (±2.5)	26.92 (±1.4)^{ab}	21.08 (±4.7)^{ab}
Mean		95.36	13.10	9.11	31.63	20.68
CV		16.36	12.91	19.46	24.47	10.28
F value		3.0359	3.4668	1.3328	2.1553	2.6398
P value		0.005225 **	0.002083 **	0.2464	0.03807 *	0.01257 *

Post-hoc multiple comparison of pruning frequency and canopy distance using two-way ANOVA ($\alpha = 0.05$)

Table 6. The impact of pruning frequency on root colonization

Pruning Frequency	Total root colonization	Arbuscular colonization	Vesicular colonization	Hyphal colonization	Mycorrhizal Hyphal colonization
No Pruning	91.78 (±3.5)^{ab}	6.46 (±1.5)^{c}	6.26 (±2.6)^{b}	11.75 (±1.5)^{c}	11.91 (±3.5)^{c}
1 - 5 Pruning	101.67 (±5.9)^{ab}	10.39 (±1.8)^{bc}	9.59 (±1.6)^{ab}	23.44 (±1.3)^{bc}	19.85 (±3..2)^{bc}
6 - 10 Pruning	77.00 (±11.3)^{ab}	17.27 (±2.1)^{bc}	12.03 (±1.6)^{b}	63.10 (±1.1)^{bc}	29.14 (±2.5)^{c}
11 - 15 Pruning	97.78 (±5.2)^{b}	10.73 (±2.7)^{ab}	5.97 (±0.8)^{a}	16.98 (±1.4)^{a}	14.86 (±3.1)^{a}
>15 Pruning	108.56 (±7.7)^{a}	20.63 (±2.9)^{a}	11.70 (±1.4)^{a}	36.31 (±1.2)^{ab}	27.67 (±3.1)^{ab}
mean	95.36	13.10	9.11	30.32	20.68
cv	22.79	11.58	56.20123	27.26	44.7763
F value	2.7134	6.4649	2.8751	4.8899	6.0813
Pr(>F)	0.0433 *	0.0004129 ***	0.03489 *	0.002637 **	0.0006401 ***

Post-hoc multiple comparison of pruning frequency using one way ANOVA ($\alpha = 0.05$)

Table 7. The impact of canopy distance on root colonization

Canopy Distance	Total root colonization	Arbuscular colonization	Vesicular colonization	Hyphal colonization	Mycorrhizal Hyphal colonization
Within canopy	107.93 (±5.4)[a]	16.50 (±2.4)	10.89 (±1.7)	34.67 (±1.25)	24.98 (±2.8)
Near to canopy	92.13 (±6.2)[ab]	13.38 (±2.0)	9.67 (±1.3)	29.51 (±1.29)	21.43 (±2.9)
Far from canopy	86.00 (±5.3)[b]	9.41 (±1.8)	6.77 (±1.1)	26.22 (±1.32)	15.64 (±2.6)
mean	95.36	13.10	9.11	30.32	20.68
cv	22.99	10.39	19.06	20.65	21.9625
F value	3.9976	3.0248	2.3208	2.552	2.8818
Pr(>F)	0.02575 *	0.04926 *	0.1107	0.08995	0.06717

Post-hoc multiple comparison of canopy distance using one way ANOVA ($\alpha = 0.05$)

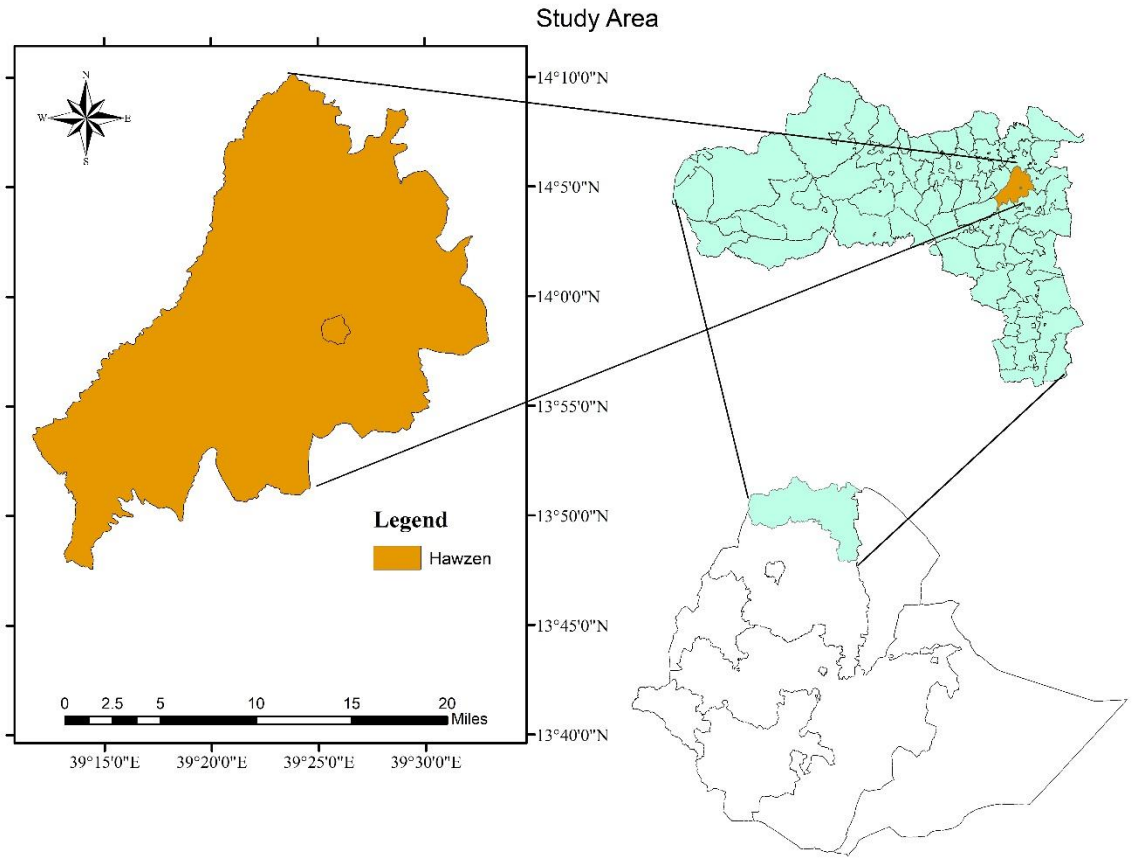

Figure 1. Location map of Study area

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