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Changes in arbuscular mycorrhiza fungi spore density and root colonization of woody plants in response to exclosure age and slope position in the highlands of Tigray, Northern Ethiopia

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The functional link between the aboveground systems and the below ground microorganisms in a plant system determines the restoration and re-establishment success of degraded ecosystems. This paper examined the arbuscular mycorrhiza fungi (AMF) spore density and root colonization in relation to slope position and age of exclosures. The first age group had less than five years old, the second age group was 5–10 years old and the third age group had 10–15 years old exclosure while the fourth age group had age between 15 and 20 years. The root and soil samples of 23 plant species that belong to 13 families from 12 sites of exclosures and grazing land were collected and analyzed using the magnified intersection and wet sieving method from the highlands of Tigray, Northern Ethiopia. . The AMF root colonization ranged from 24% to 96%. The lowest colonization was observed from plant species that belong to the grazing land, and the highest were from plant roots in exclosures. The spore density was between 30 and 2980 of 100 g^{-1} of dry soil with the lowest from the grazing lands and the highest was from exclosures of middle slope position. *Glomus* was the dominant AMF genus found in all soil from both land uses. A significant difference in spore density ($p < 0.05$) was observed between slope position and age of exclosures. AMF root colonization positively correlated ($P < 0.05$) with spore density. Exclosures of middle slope showed high spore density and root colonization and it increased significantly with increasing age of exclosures. The presence of abundant AMF spore in exclosures indicated the role exclosures played in the restoration of ecosystem health. Forestland restoration through exclosures could facilitates the survival of planted and regenerated plants by providing enough AMF inoculum in the restored ecosystem. AMF spore density and colonization should be considered as an indicator of restoration in measuring success of restoration in the drylands.

1. Introduction

The idea of exclosure involves a protection system, exclusion of the degrading agent, to allow the lands to re-establish itself through natural succession process. Exclosures are areas that exclude human and livestock interference for rehabilitation of degraded lands in the drylands (Seyoum et al., 2015). Degraded lands that almost lost their production potentials left for nature based rehabilitation, and if properly managed and rehabilitated through exclosure system, allow native vegetation to restore. Many case studies conducted in highlands showed that exclosures are effective in enhancing composition, diversity, and density of vegetation (Yayneshet et al., 2009). Species that disappeared long time ago restored following the establishment of exclosures. For instance, species that could not be observed for many years in some parts of eastern Tigray, namely *Olea europaea* subsp. *cuspidata* and *Juniperus procera*, reappeared, densities and diversities of the flora, particularly of grasses, and fauna increased, soil erosion decreased and even dead springs started to flow after exclosures were established (Birhane et al., 2007).

The restoration and re-establishment of degraded ecosystems should include not only the aboveground systems but also the below ground microorganisms which are linked functionally with plants (Li et al., 2007). The success of any ecosystem restoration

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efforts are likely to depend on the establishment of mycorrhizas, and Arbuscular Mycorrhiza Fungi (AMF) should receive special consideration in restoration of degraded ecosystem (Wubet et al., 2003). Owing to the multiple beneficial effects on plant performance and soil health, AMF are essential for the restoration and re-establishment of the vegetation in degraded ecosystems (Dhillion and Gardsjord, 2004). AMF are more commonly distributed than other types of mycorrhizal associations (Smith and Read, 2008) and are keystone organisms that form an interface between soils and plant roots, and are sensitive to changes in soil and plant environment.

Disturbance can affect the incidence of AM fungi in both agricultural and natural ecosystems. Land use change and/or disturbance of soil can reduce mycorrhizal infection and several factors may be responsible. Soil disturbance negatively affect the functionality of AMF (Trejo et al., 2016). There may be effects of tillage on root growth affecting the degree of root colonization by mycorrhizal fungi (Borie et al., 2006). The influence of grazing on soil nutrient availability and host plant productivity may cause inconsistent effects on AMF community composition and structure (Bai et al., 2013). Grazing of pasture grasses in the field has been found to affect the amount of root length infected by decreasing root length per unit volume of soil (Yang et al., 2013). Grazers also influence allocation to AMF morphological structures by changing soil nutrient condition through direct inputs of N and P in dung and urine deposition (van der Waal et al., 2011; Schnyder et al., 2010). AMF differ in their response to the mineral environment of the soil (Brundrett, 2004).

One way by which plants can potentially enlarge ecosystem productivity and stability is by forming mycorrhizal associations (Eriksson, 2001). Plants are most likely to form associations with and benefit from mycorrhizal fungi under situations in which availability of one or more soil nutrients, including water, is low (Smith and Read, 2008). Tropical savanna soils have been eroded and poor in nutrients resulting to reduced plant productivity (Pimentel, 2006). AMF are of particular significance to the plant in soils that are nutrient poor (Jeffries et al., 2003). Moreover, AMF serve as sensitive indicators of ecological soil quality if they respond to environmental variation in a predictable way (Verbruggen et al., 2012). Information about species composition of AMF community appears important to recognize mycorrhizal function in the ecosystems. It is evident that AMF are essential for the functioning of terrestrial ecosystems. Therefore, understanding the impact of land use on AMF abundance in tropical soils is important. Forest restoration in protected exclosures has become a widespread practice to fight land degradation in the highlands of Northern Ethiopia (Mekuria and Aynekulu, 2013). Exclosures have been implemented in grazing areas for the past decades in Ethiopia and have been effective in regenerating natural vegetation, controlling soil erosion and increasing soil fertility (Baudron et al., 2015). Despite this effort, studies that evaluate the effectiveness of different exclosure habitat types to restore degraded soils and vegetation, their role to the enhancement of microorganisms in soils are lacking. Besides, it is hardly possible to find studies that evaluate the benefits of exclosures to restore arbuscular mycorrhiza fungi (AMF). This paper aimed at investigating the spore density and root colonization of AMF in relation to land use, age and slope positions of the exclosures and the dynamic with soil nutrients. The research questions answered in this paper were; is conversion of free grazing land to exclosure increased AMF spore density in rhizosphere soils and colonization of plant roots? Does age of exclosures at different elevation gradient influence the availability of AMF spore density and percent of root colonization? What is the relationship of AMF spore density and root colonization in relation to the available nutrients?

2. Materials and methods

2.1. Study area

The study was conducted in the highlands of Tigray region, Northern Ethiopia in four zones and four districts (Fig. 1). There were 12 sites having exclosures and a grazing land. Exclosure is a method of rehabilitating land by protecting an area from the interference of animals and human encroachment for limited period of time, depending on site capacity and vegetation re-establishment (Seyoum et al., 2015). The grazing lands are areas open for grazing continuously by livestock. The exclosures were divided in to four age classes and three slope positions to study the age, slope position and land use effect on AMF and soil physico-chemical properties. The first age group had less than five years old exclosure with triplicates sites, the second age group was 5–10 years old exclosure with triplicates sites and the third age group had 10–15 years old exclosure while the fourth age group had age between 15 and 20 years. All sites have tropical semi-arid climate. The altitude of the study sites ranged from 2232 to 2937 m.a.s.l. The rainy season usually occurs between June and September (Fig. 2) and the growing season varies between 90 and 120 days. The highest rainfall is in July and August that ranges between 162 and 228 mm. The maximum temperature is in the months of May and June (Fig. 2).

Luvisols (Alfisols), Regosols (Entisols), Cambisols (Inceptisols) and Calcisols (Aridisols) were major soil groups in the study area (WRB, 2006). The study sites were dominanted by Luvisols (Alfisols) and Cambisols (Inceptisols). *Acacia etbaica, Acacia seyal* (Del.), *Becium grandiflorum* (Lam.) Pichi-Serm. *Euclea racemosa* subsp. *schimperi* (A. DC.) Dandy and *Maytenus arbutifolia* (Hochst. ex. A. Rich) Wilczek were the common woody vegetation species in exclosures and grazing lands (Mekuria and Aynekulu, 2013). The exclosures are mainly covered by trees, shrubs and the ground by grass. The life form of woody plants in the exclosures were 35.1% trees and 39.73% were shrubs while the rest were woody herbs and climbers. The life forms in the open grazing lands were 83.37% shrubs and 5.7% were trees while the rest were woody herbs; shrubs significantly outnumbered the trees in the exclosures. The abundance of the naturally regenerated woody plants in the exclosures was 91.03% while 8.9% was found artificially planted but no planted seedling was observed in the open grazing lands. The abundant species in the exclosures was composed of naturally regenerated species.

Mixed farming system that integrates crop and livestock was the main means of livelihood. The major land uses were cultivated land (9–33%), forestland (3–58%), exclosure (3–16%), communal grazing land (6–39%) and others (20–41%) of the total area (Mekuria and Aynekulu, 2013). The main crops cultivated were Teff (*Eragrostis teff* (Zucc.) Trotter), Bread wheat (*Triticum aestivum*), Maize (*Zea mays* L.), Sorghum (*Sorghum bicolor*), Barley (*Hordeum vulgare*), and Faba bean (*Vicia faba*).

2.2. Experimental design

The role of exclosure on soil physico chemical properties, AMF spore density and root colonization, were studied by taking rhizosphere soils and roots under the different slope positions and ages of exclosures and adjacent grazing lands as controls. The assumption were prior to establishment of exclosures, the control sites had similar conditions as the exclosures were established on the same communal grazing lands which were used for livestock grazing (Mekuria and Aynekulu, 2013). The experiment was composed of 12 experimental units with four treatments (age groups) replicated three times in the two land uses. In the entire

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