Role of soil microbes from remnant Church Forest to assist seedling establishment of native tree species in a degraded land

(劣化地での在来樹種の実生定着における遺された「教会の森」由来の土壌微生物の役割)

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The United Graduate School of Agricultural Sciences
Tottori University, Japan

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A dissertation submitted to
The United Graduate School of Agricultural Sciences, Tottori University
in partial fulfillment of the requirements for the Degree of Doctor of Philosophy
in Global Arid Land Science

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List of Abbreviations and Acronyms

°	Degree
%	Percent
˚C	Degree Celsius
Avail.	Available
AG	*Albizia gummifera*
ANOVA	Analysis of variance
bp	base pair
C	Carbon
CM	*Croton macrostachyus*
cm	Centimeter
C/N	Carbon to nitrogen ratio
CEC	Cation Exchange Capacity
D	Simpson diversity index
df	Degree of freedom
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
g	gram
H’	Shannon diversity index
ha	Hectare
ITS	Internal transcribed spacer
JP	*Juniperus procera*
L	Liter

M	Mega
m	Meter
m.a.s.l	Meter Above Sea Level
Mg	Mega gram
mg	milligram
mm	millimeter
N	Nitrogen
n	Number of individuals
NMDS	Multidimensional scaling
ns	Not significant
OTU	Operational Taxonomic Unit
P	Phosphorus
p	Probability
p-value	Provability value
PCR	Polymerase chain reaction
pH	power of Hydrogen
PerMANOVA	Permutational multivariate analysis of variance
QIIME	Quantitative Insights Into Microbial Ecology
RNA	Ribonucleic acid
R	R statistical software
R/S	Root to shoot ratio
r^2	Coefficient of Correlation
16s rRNA	16S ribosomal Ribonucleic acid
TN	Total Nitrogen

SAS	Soil aggregate stability
SD	Soil Depth
sd	Standard deviation
SE	Standard error
SOC	Soil Organic Carbon
ρd	Bulk density
μ	micro

Chapter 1

General Introduction

1.1 Background

1.1.1 Deforestation and land degradation

Human activity has become the main factor of global ecosystem change (Deng et al., 2016; Smith et al., 2016; Curtis et al., 2018; Wolff et al., 2018). Deforestation and unsustainable land management practices, escalated by climate change, has led to land degradation (Wolff et al., 2018). Land degradation is a major global problem affecting all terrestrial biomes and agro-ecologies (Le et al., 2016; Mekuria et al., 2018). Land degradation is a key driver of ecosystem function and services loss (Wolff et al., 2018). The loss of vegetation cover and soil nutrients reduce the productive potential of soil and affecting global food security (Rojas et al., 2016). Land degradation affects around 2 billion hectares of land and 25 % of these land area considered degraded worldwide (Muñoz-Rojas et al., 2016). Land degradation also treats nearly 3.2 billion people globally (Le et al., 2016).

Population increment and the growing need for expansion of agricultural land and associated activities, such as deforestation, overgrazing, and improper agricultural practices are the main factors causing land degradation (Nunes et al., 2012; Araujo et al., 2014; Meshesha et al., 2014; Singh, 2015). Land degradation due to deforestation significantly caused a loss of vegetation cover, biodiversity, soil fertility and productivity (Figure 1-1a and b). Regardless of the complexity of deforestation causes, it estimated to attributed 27% of forest loss from 2001 to 2015 globally (Curtis et al., 2018). This loss primarily occurring in the tropics (FAO, 2020), in relation to agricultural expansion (Figure 1-1b), which contributed to 70–95% of forests lost in the region (Hosonuma et al., 2012). Similarly, in sub-Saharan Africa, deforestation is five times larger than the global average (Olsson et al., 2019), and responsible for the 22% of the total global cost of land degradation (Nkonya et al., 2016).

Like other tropical countries, in Ethiopia, land degradation is the most serious threat that challenging agricultural production, food security, and natural resource conservation of the country. Deforestation, forest burning, and expansion of cultivation lands are the main contributors to the widespread of land degradation in the country (Figure 1-1c and d). This has led to a sever soil erosion (i.e. water), which is the most common form of land degradation in the country (Fenta et al., 2017). Soil erosion also considered the most important environmental problem (Hurni et al., 2016; Fenta et al., 2017; Haregeweyn et al., 2017). Recent estimates indicated that soil erosion, is very severed and cause loss of the productive top layer of soils

(Haregeweyn et al., 2017). The loss of this active layer of the soil significantly affects the soil nutrient (Tuo et al., 2018), microbes (Xiao et al., 2017), vegetation, soil seed-bank, and seedling recruitment (Garcia-Fayos et al., 2010; Shang et al., 2016) and the recovering potential of degraded lands.

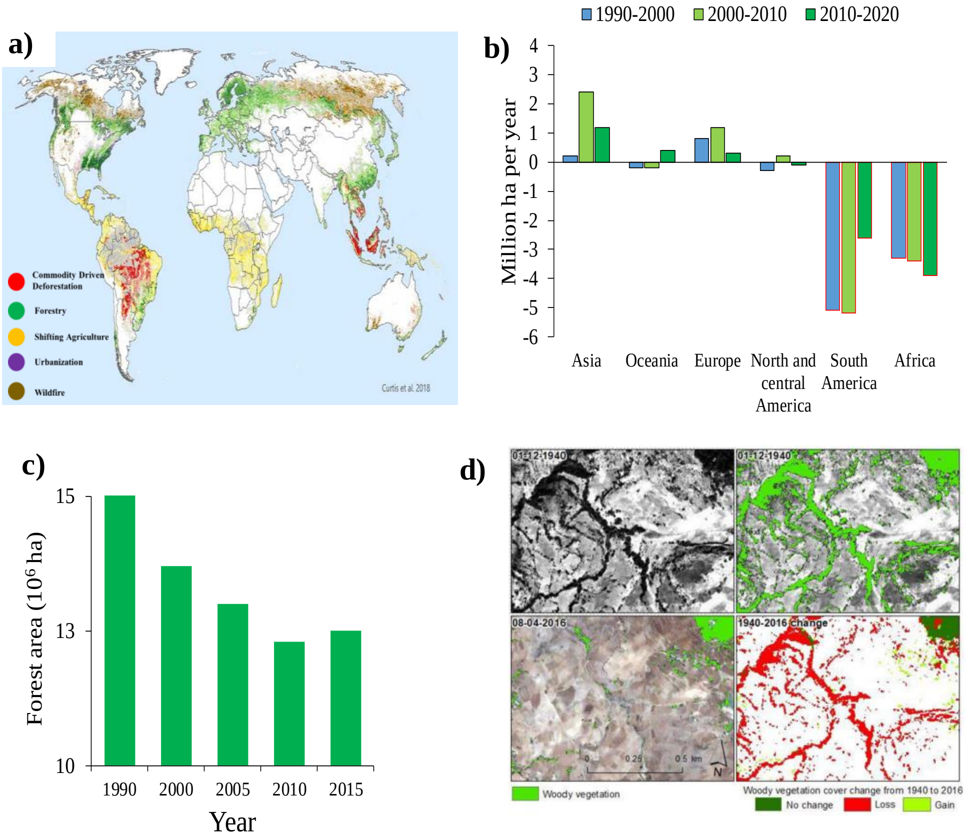

Figure 1-1. Extent of global land degradation (a), deforestation (b) and annual forest loss in between 1990-2020 (c) and 1990-2015 in Ethiopia (d). Source: a (Curtis et al., 2018), b (FAO, 2020), c (FAO, 2015) and d (Frankl et al., 2019)

1.1.2 Ecological restoration of degraded lands

In many regions of the world, various methods of restoration are necessary to rectify degraded ecosystems (SER, 2004). Ecological restoration refers to the process of re-establishing the structure, productivity, and species diversity of the degraded ecosystem (Lamb and Gilmour, 2003; McDonald et al., 2016). This process includes management methods, which assisting the recovery of a natural or semi-natural ecosystem on degraded land (SER, 2004). Approaches in restoring the lost function of degraded ecosystems strongly depend on the original state of the ecosystem or damage level, the desired outcome, period of time frame, and cost (Chazdon, 2008).

Ecological restoration aims to restore a degraded ecosystem to its intact state (Figure 1-2, arrow 1) such as historic production, species composition, and diversity referred as "*sensu stricto"*(Bongers et al., 2006). Depending on the damage level previous land use, restoring the vegetation of a degraded site to pre-disturbance natural state depends on factors including availability of persistent seed in soil (i.e. seed bank), arrival of seed, and presence of safe site (Lemenih and Teketay, 2004). These key factors control the rate of the restoration and recovery of plant species, composition, structure, and function on the restored ecosystems (Aerts et al., 2007). In many cases, a restored ecosystem may not match the composition and structure of the original ecosystem because of irreversible changes in the degraded ecosystem conditions including species, soil (physical, chemical, and biological) and climate (Bongers et al., 2006; Wassie Eshete, 2007; Tuo et al., 2018). Therefore, an ecosystem could be restored partially to an intermediate level of the original ecosystem (Bongers et al., 2006; Wassie Eshete, 2007; Chazdon, 2008; Stanturf et al., 2014) (Figure 1-2, arrow 2). This approach also called "rehabilitation", which restored some form of ecosystem functionality and a substantial proportion of the native biota found in reference ecosystem (Stanturf et al., 2014). In this process usually integrated purposely selected non-native or exotic species to full fill the desired ecosystem functions (Stanturf et al., 2014). Therefore, the rehabilitated degraded ecosystem will be functioning in the same way to the reference system and contain similar but not necessarily the same vegetation (Chazdon, 2008).

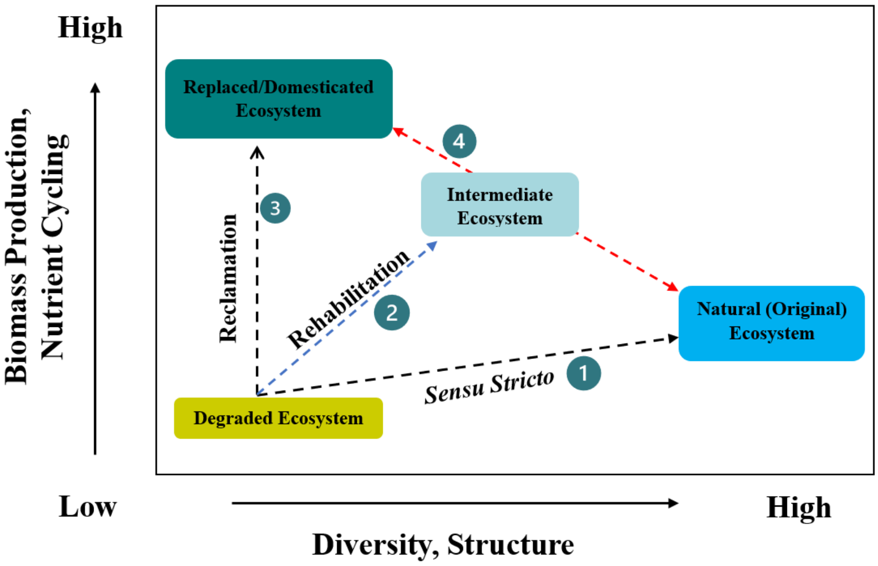

Figure 1-2. Approaches in ecological restoration. Arrows represents options 1) degraded ecosystem to restoration, 2) rehabilitation, 3) reclamation and 4) domestication to natural restoration

In fact, a degraded site has some self-recovering potential. However, in many aspects the biotic and abiotic environmental conditions in degraded lands are poor for self-regenerating (Lemenih and Teketay, 2004). Therefore, a restoration approach such as reclamation is a viable option for restoring some levels of biodiversity and ecosystem services, where impoverished soil, limited establishment, and growth of native vegetation (Stanturf et al., 2014). This process also intends to increase the productivity and improving the economic benefits from a degraded ecosystem (Figure 1-2, arrow 3). Several recent studies have revealed that plantation of exotic fast growing species have been successfully used to improve soil nutrient status and to stimulate native vegetation establishment on degraded lands (Jeddi and Chaieb, 2012; Ramírez et al., 2014; Tesfaye et al., 2015). Particularly, species with traits including nitrogen fixation, fast growth, and high adaptability to harsh environment introduced to promote ecological restoration for late successional native species (Ramírez et al., 2014; Thijs et al., 2014). However, there are concerns about planting monocultures of exotic species (i.e., *Conifer*, *Acacia*, and *Eucalyptus*) in restoration sites and their adverse environmental effects on biodiversity, soil and water conditions (Xu et al., 2018; Feng et al., 2019).

1.1.3 Ecological restoration in Ethiopia

Ethiopia is agrarian country, where agriculture is the backbone of the economy and provides major occupation for more than 85% of the country's population. Consequently, deforestation because of agricultural land expansion and land use change altered the land scape of the country (Lemenih and Teketay, 2004; Nyssen et al., 2004; Frankl et al., 2019). Recent estimates reported that crop lands in the highlands holds 80% of the land mass, whereas natural forest covers less than 3% (Moges et al., 2020). These forest are isolated and fragmented found in a small patches and in accessible areas. For instance, forests around the Ethiopian Orthodox Tewahido churches and monasteries (Wassie et al., 2009a; 2009b), also called "Church Forests" consist of some of the remnants of the climax tree species and potentially served as source of seed, propagule, and site for reference (Wassie et al., 2009b; Abiyu et al., 2016; Aerts et al., 2016). Due to prolonged agricultural history of the country, particularly in the highlands, land degradation has been causing the most severe problem on the agricultural production and food security (Nyssen et al., 2004; Jacob et al., 2014). Therefore, conserving and restoring these degraded ecosystems is the priority of the country to enhance ecosystem services and functions, protect biodiversity, and contribute to climate resilience, food security and landscape sustainability.

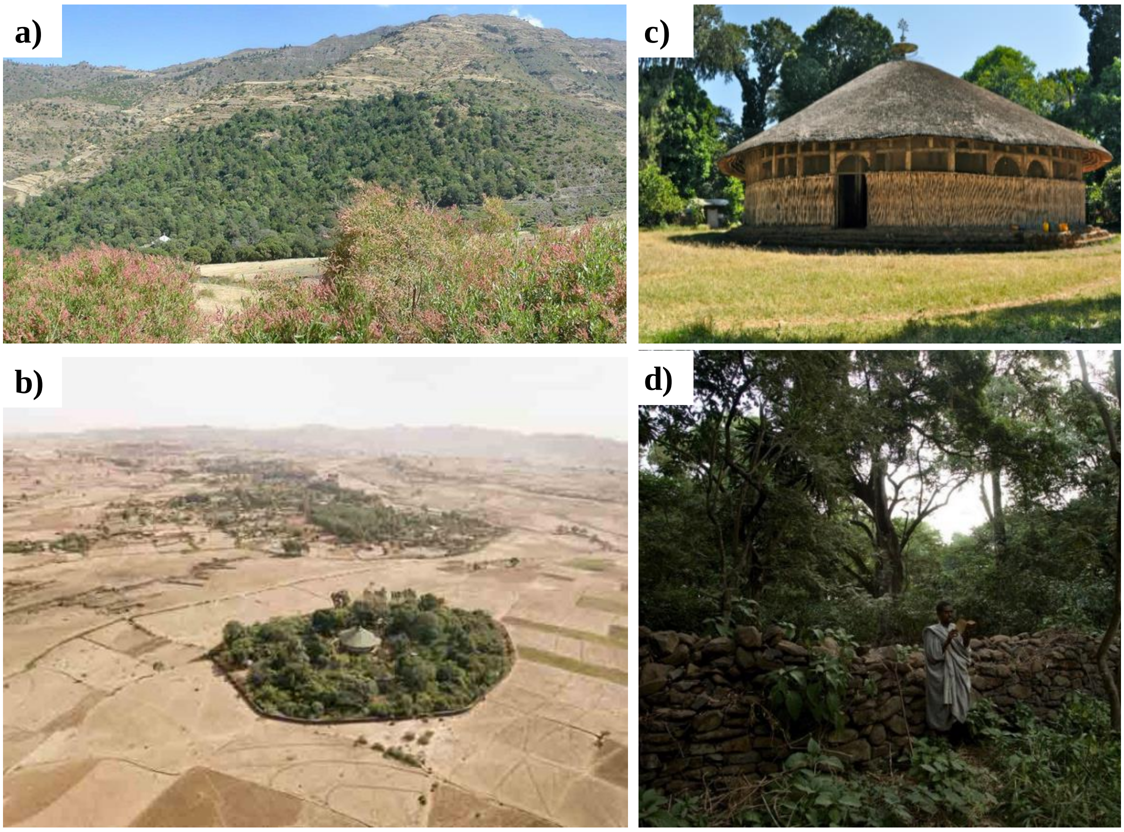

Figure 1-3 Church Forest in the highland of Ethiopia. (source: a (Aerts et al., 2016), b and c (Abbott, 2019), d (Church Forest in Aba Gerima, Ethiopia))

Since in 1970’s soil and water conservation interventions have been proposed and implemented to curb land degradation (Hurni et al., 2010; Hurni et al., 2016; Sultan et al., 2018). These include terracing, afforestation and exclosure establishment (Damene et al., 2013; Mekuria et al., 2018). Particularly, exclosures have been among the top priority’s measure in degraded hillside areas. The concept of exclosure comprises a protection system, exclusion of the disturbance agents (Yirdaw and Monge, 2018); restricted access for vegetation (Mengistu et al., 2005) and allow the lands to recover itself through natural succession process (Birhane et al., 2017). In this regard, several studies have confirmed the significance of exclosures in revegetation and soil nutrient enhancement (Wassie et al., 2009; Mekuria et al., 2017; Mekuria et al., 2018). On the other hand, studies also reported most of the exclosure lacks viable seeds from the soil to restore the original forest, as a result of prolonged cultivation and severe degradation after the conversion (Aerts et al., 2007). Along with, limited distance seed dispersal nature of the this forest tree species, exclosure have faced a serious scarcity of native tree species regeneration, in particular, for areas where seed sources (i.e., Church Forest) are fragmented and isolated in a landscape (Wassie et al., 2009a). For this reason, in the highlands, the restoration effort integrates soil and water conservation measures and seedling plantation to facilitate the ecosystem restoration (Damene et al., 2013; Mekuria et al., 2017). Many ecologists approved that seedling panting is crucial to accelerate native tree regeneration in exclosures (Mengistu et al., 2005; Aerts et al., 2007; Abiyu et al., 2016).

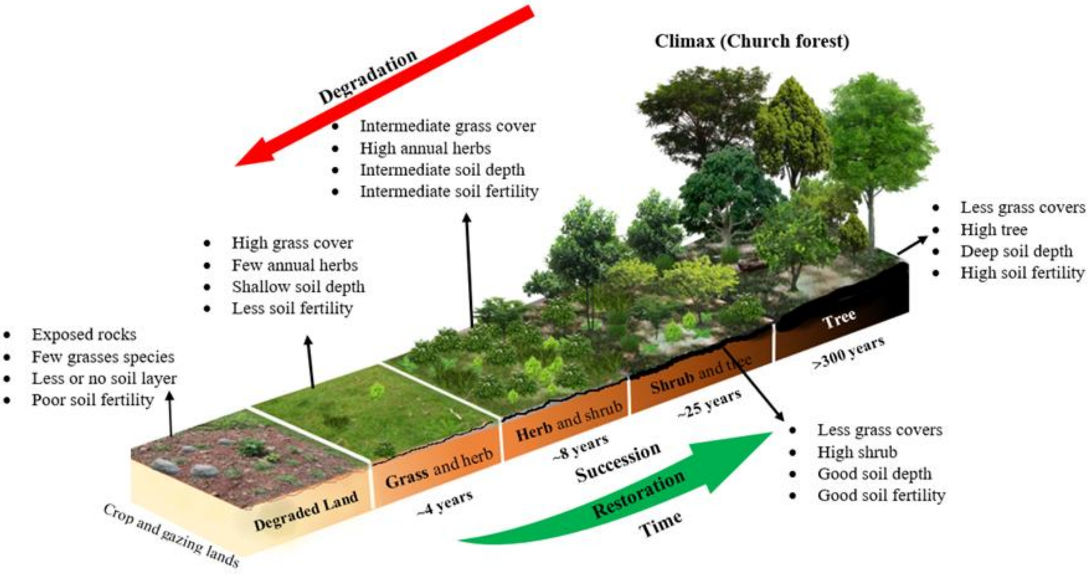

Figure 1-4**.** Restoration of degraded land in Ethiopia

1.1.4 Seedling establishment in degraded lands

Seedling establishment is an ecological processes of seed germination and seedling establishment (Wassie Eshete, 2007). This process mainly controlled by the different factors including availability of persistent seed and hospitable microsite condition (Feyera Senbeta and Teketay, 2001; Abiyu et al., 2016).

1.1.4.1 Viable seed availability

The possible sources of viable seeds for seedling establishment are from seed rain, soil seed bank and seed dispersal in most ecosystems (Wassie Eshete, 2007; Abiyu et al., 2016). Most of the seed rain depends on seed dropping from local mother trees and immigrate by biotic and abiotic dispersed agents (Bürzle et al., 2017). Therefore, seed rain can be influenced by these agents and the change in landscape characteristics (San-José et al., 2019). Moreover, seed rain limited by change reproduction capacity of tree as a result of forest fermentation, loss of pollinators and climate variation (Munzbergova and Herben, 2005). The availability of seed in soil seedbank also depends on the potential of the species to maintain viable soil seed bank.

Presence of viable seed in soil seed bank considered as a memory of the past plant community and capable of replacing the adult plant (Lemenih and Teketay, 2004). However, in degraded lands the availability of seed from these sources are limited (Bertacchi et al., 2016). Specifically, studies in the Afromontane region of Ethiopian highlands reported that the soil seed banks in degraded lands are poor and dominated by grasses and annual herbs, which potential restricted the seedling establishment (Teketay and Granström, 1995; Kebrom and Bekele, 2000; Lemenih and Teketay, 2006). Therefore, site preparation and assisting seedling establishment has been recommended to actively restore this degraded ecosystem.

1.1.4.2 Availability safe site

Availability of safe sites for seedling establishment are key for successful restoration (Bürzle et al., 2017). Beside availability of persistent seeds, the occurrence of a safe site is the most limiting factor for seedling establishment and survival (Aradottir and Halldorsson, 2018). The safe site requirements of plants are species dependent and differ among seedling life stages. In most of the degraded lands the extreme environmental conditions including high temperature, depleted nutrients, poor moisture and destructive wind are quite uninhabitable for seedling establishment (Bertacchi et al., 2016). In such cases, seedlings often needed additional facilitation that assist the modification of the physical microenvironment. Both abiotic: topographic shelter, rocks and stones (Bürzle et al., 2017) and/or biotic: nurse plant (Padilla and Pugnaire, 2006; Aerts et al., 2007), microbial symbiosis (i.e. bacteria and fungi) (Lin et al., 2015; Singh, 2015; Wubs et al., 2016) facilitators are commonly used to create favorable conditions for seedling establishment (Aradottir and Halldorsson, 2018). For instance, in recent years, restoration ecologists have given more effort on the impact of nurse plants involved in facilitation role of the other plant under their canopy (Lu et al., 2018; Tulod and Norton, 2019). Studies has been confirmed that nurse plants can create hospitable conditions for the growth and survival of other plants beneath their canopy zones (Padilla and Pugnaire, 2006; Aerts et al., 2007; Lu et al., 2018). O'Donnell et al. (2020) reported nurse shrub (*Juniperus ashei)* improved the microsite condition and significantly contributed for the growth and survival of Oak seedling in eastern U.S. Similarly, Aerts et al. (2007) reported that pioneer shrubs had great impact on *Olea europaea* seedling establishment in exclosures by improving soil-water condition, shading from intensive solar radiation and protection from browsers in the highland of Ethiopia. Therefore, nurse shrubs can be seen as an ecosystem services providers and also suggested by many studies to be actively used to restore degraded lands. On the other hand, microbial inoculation used as a facilitator to enhance seedling establishment in degraded lands (Singh, 2015; Lance et al., 2019; Neuenkamp et al., 2019). Several studies have been demonstrated that microbial inoculation could increase the soil nutrient availability (Wubs et al., 2016; St-Denis et al., 2017), soil moisture (Kannenberg and Phillips, 2017; Muñoz-Rojas et al., 2018), soil aggregation (Rillig et al., 2015; Rashid et al., 2016), pathogen protection (Wehner et al., 2010) during seedling establishment in harsh environments.

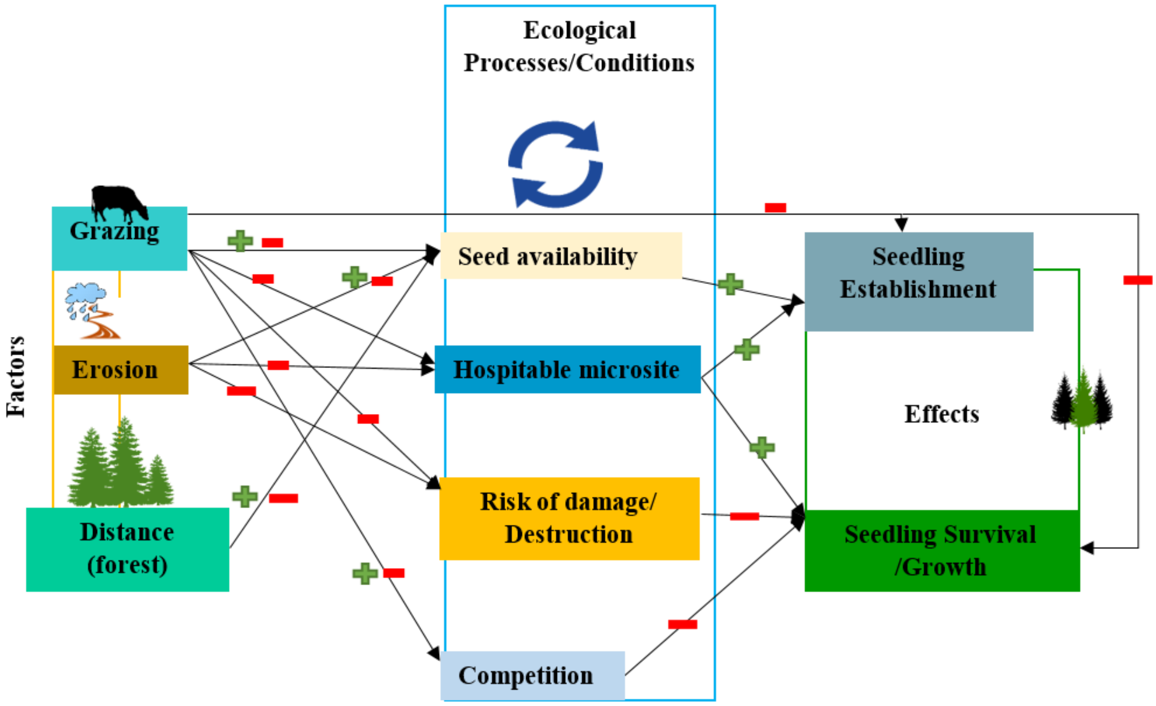

Figure 1-5. Schematic representation of interactions of factors and ecological processes controlling seedling establishment, survival and growth of native tree species in the degraded lands of Ethiopia (highland). Arrows with sign (+) and (-) indicated expected positive, and negative relations, respectively (the figure is modified and adopted from Wassie Eshete (2007)).

1.2 Problem statement

In Ethiopia, land degradation is main challenge for the agriculture production and ecological processes. Climate change, population increment, deforestation and land use change has been significantly affecting the soil resource and ecosystem sustainability. Therefore, to avert the land degradation various interventions (i.e., restoration) have been implemented since 1970's. However, information on key ecological indicators such soil organic carbon and total nitrogen stocks still scares to prioritize and design evidence-based land management options.

Moreover, as a result of sever soil erosion and loss of the active soil layer achieving restored ecosystem function and diversity through re-establishment of native tree species (Aerts et al., 2007) has proved challenging due to the lack of a native soil community in degraded lands.

Indeed, soil microbes in combination with plant species play a crucial role in restoring degraded lands (Ambrosini et al., 2015; Neuenkamp et al., 2019). In this regard, very little information exists about the source of native inoculum for the successful restoration of lost microbial community functions in degraded landscapes. Whereas small patches of natural forest, called "Church Forests," exist around Ethiopian Orthodox Tewahido churches and monasteries, and these constitute the last remnants of the original forest cover, having been conserved for more than a century (Bongers et al., 2006; Wassie et al., 2010; Abiyu et al., 2016). These Church Forests have social, spiritual value, and a foci of biodiversity used for seed source and germplasm conservation site for native flora (Wassie et al., 2010; Berhane et al., 2013; Abiyu et al., 2016; Aerts et al., 2016). Similarly, soils in Church Forests are assumed to host microbiomes that are beneficial for the ecosystem functioning. However, there is a lack of studies characterizing the role of microbial communities or evaluating the inoculum potential and the effects of microbes from Church Forests on the early stages of native tree establishment.

1.3 Objectives of the study

The overall objective of this research is to contribute for the assisted-restoration of native trees in degraded lands through using Church Forest soils as a source of native microbes. The specific objectives were 1) to assess land degradation using the SOC and TN stocks in different land use, topographic position and three agro-ecosystems of the Upper Blue Nile basin; 2) to assess the soil microbial potential of soils from Church Forest and its effect on seedling growth of *Olea europaea* and *Albizia gummifera* under glasshouse condition and 3) to evaluate the role soil from Church Forest in assisting seedling of *Olea europaea* establishment under a degraded land conditions

1.4. Description of the study site

1.4.1 Location and climate

The study was conducted in three different agroecosystems of the Upper Blue Nile basin, Ethiopia (Fig. 6). Guder (11°0′35.13″N, 36°56′7.97″E), Aba Gerima (10°45′53.09″N, 36°16′19.11″E), and Dibatie (11°39′27.26″N, 37°30′14.21″E) represented the highland, midland, and lowland agro-ecosystems, respectively (Table 1). According to (Mekonnen, 2016), the four dominant soil types (in the FAO classification system) of the study area are Acrisols, Leptosols, Luvisols, and Vertisols (Table 1).

In the Koppen–Geiger classification (Peel et al., 2007) the climate is characterized as subtropical oceanic highland at Guder, humid subtropical at Aba Gerima, and tropical wet-dry at Dibatie. The rainfall pattern is unimodal and mostly occurs from June to September at all sites (Figure 7). The mean annual rainfall was 1022, 1343, and 2454 mm at Dibatie, Aba Gerima, and Guder, respectively (Yibeltal et al., 2019). Mean annual temperature varies from 25 to 32 °C at Dibatie, from 13 to 27 °C at Aba Gerima, and from 9.4 to 25 °C at Guder (Nigussie et al., 2017; Ebabu et al., 2019).

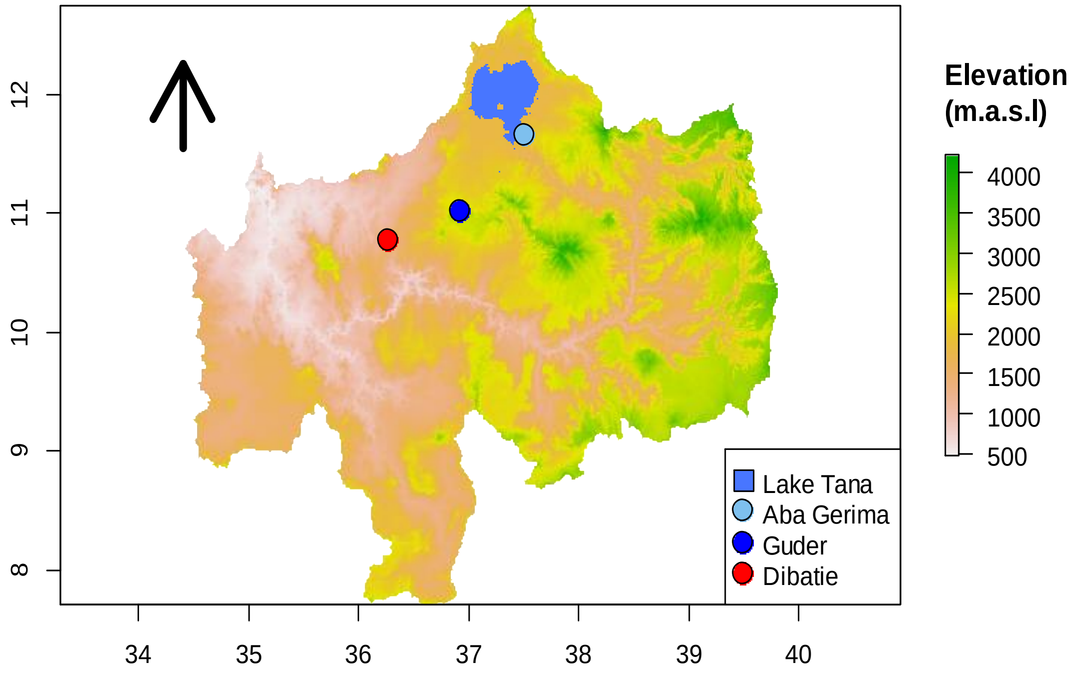

Figure 1-6. Location of the study sites.

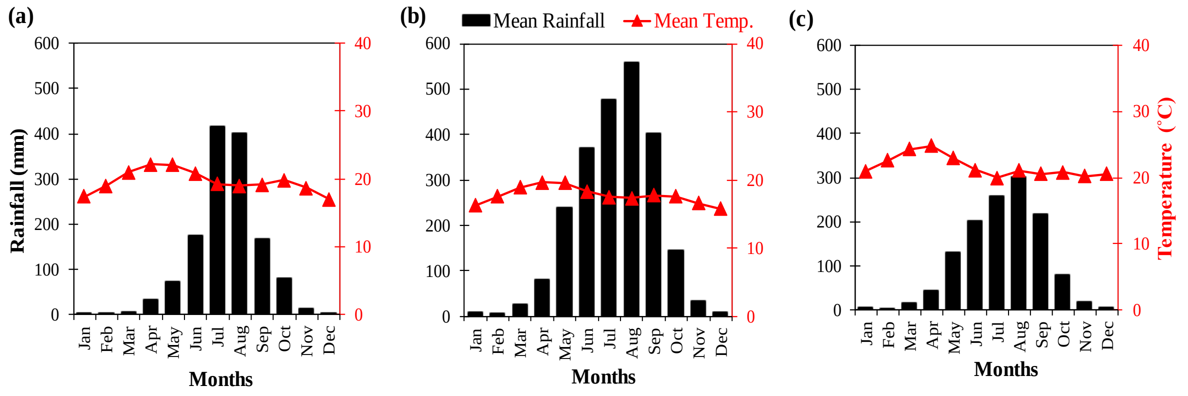

Figure 1-7. Climograph of Guder (a), Aba Gerima (b), and Dibatie (c) from 1999 to 2017.

1.4.2 Vegetation and farming system

The native tree and shrub species common at Guder are *Acacia abyssinica*, *Albizia gummifera*, *Croton macrostaches*, *Combretum molle*, *Cordia africana*, *Schefflera abyssinica*, *Dovyalis abyssinica*, and *Entada abyssinica*. Those at Aba Gerima are *A. gummifera*, *Bersama abyssinica*, *Calpurnia aurea*, *Croton macrostaches*, *Olea europaea*, *Ficus thonningii*, and *E. abyssinica*. At Dibatie *Acacia negrii*, *Acacia sieberiana*, *Ficus sycomorus*, *Terminalia brownii*, *Terminalia schimperiana*, and *Oxytenanthra abyssinica* are common in the bushlands. *A. decurrens* at Guder and *Eucalyptus camadulensis* at Aba Gerima are the dominant exotic tree species planted as woodlots for fuelwood, charcoal, and construction wood production. Clear felling (Guder) and coppice management (Aba Gerima) are the common plantation management practices. The rotation period of the plantations at Guder and Aba Gerima is 3–5 and 5–7 years, respectively.

Rainfed, subsistence-based and mixed farming (crop cultivation and livestock rearing) is the main agricultural practice at the study sites (Nigussie et al., 2017). At Guder, teff (*Eragrostis tef*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) are grown. At Aba Gerima, teff, finger millet (*Eleusine coracana*), wheat, and maize (*Zea mays*) are cultivated. At Dibatie, maize, teff, sorghum (*Sorghum bicolor*), and groundnut (*Arachis hypogaea*) are the major food crops (Ebabu et al., 2019; Yibeltal et al., 2019a)

Table 1-1. Site characteristics of study watersheds in the Upper Blue Nile basin

Site characteristics	**Site (watershed)**		
	Guder (Akusty)	**Aba Gerima (Kecha)**	**Dibatie (Bekafa)**
Longitude, latitude	11°0′35.13″N, 36°56′7.97″E	10°45′53.09″N, 36°16′19.11″E	11°39′27.26″N, 37°30′14.21″E
Area (ha)	343	426	246
Elevation (m a.s.l.)	2500–2800	1900–2200	1400–1700
Slope gradients (°) [a]	0–32	0–36	0–21
Annual mean temperature (°C) [b]	9.4–25	13–27	25–32
Rainfall (mm yr^{-1}) [b]	1951–3424	895–2037	850–1200
Agro-ecology [c]	oceanic subtropical	humid subtropical	tropical wet-dry
Soil parent material [d]	Basalt (Quaternary)	Basalt (Oilgo pilocene)	-
Major soil types [e]	Acrisols and Leptosols	Leptosols and Luvisols	Luvisols and Vertisols
Primary soil texture [a]	clay loam	clay	clay
Sand, silt and clay (%) [e]	30, 40 and 30	15, 30 and 55	25, 19 and 56
Selected soil properties			
pH (water)	4.2–6.5	4.7–6.8	5.8–7.4
Electric conductivity (dS m^{-1})	0.01–0.11	0.01–0.12	0.02–0.19
Cation exchange capacity (cmol kg^{-1}) [e]	21.4–65.7	23.8–26.8	23.2–48.8
Land-use types (Area, ha)	bushland (58.8), cropland(106), grazing land (47.1), plantation forest (116.5)	bushland (46.5), cropland (220), grazing land (14.6), plantation forest (38)	bushland(37.6, cropland (151), grazing land (55.3)
Dry biomass (tones ha^{-1} yr^{-1}) [f]	7.14	6.17	6.94
Cropland (teff)	3.9	3.08	7.9
Grazing land	7.14	6.17	6.94

[a] (Yibeltal et al., 2019a). [b] Weather data (1999–2015) was obtained from (Ebabu et al., 2019). [c] Koppen–Geiger classification (Peel et al., 2007).[d](Poppe et al., 2013), [e] (Mekonnen, 2016). [f] Dry biomass data was obtained from KAKENHI project (average from 150 plots [1 m × 3 m], 2016–2017).

1.5 Organization of the thesis

This thesis arranged into chapters (Figure 1-8). Chapter 1 presents the introduction sections (Background, problem statement, objectives, and study sites) of this thesis. It introduces the causes and impacts of deforestation and land degradation, ecological restoration approaches based on previous literature, on-site view, and the rationale of the study. Chapter 2 assess the land degradation status using soil organic carbon and total nitrogen in relation to land use and topographic position in the three agro-ecosystems (highland, midland and lowland), which represents different ecological, socioeconomic settings of the basin. Chapter 3 assess the soil microbial potential of soils from Church Forest and its effect on seedling of two selected native tree species under glasshouse condition. Chapter 4 evaluate the role soil microbial from Church Forest in assisting seedling of *Olea europaea* establishment under a degraded land conditions. The last chapter (chapter 5) describes the general conclusion and recommendations based on the key findings of chapter 2, 3 and 4.

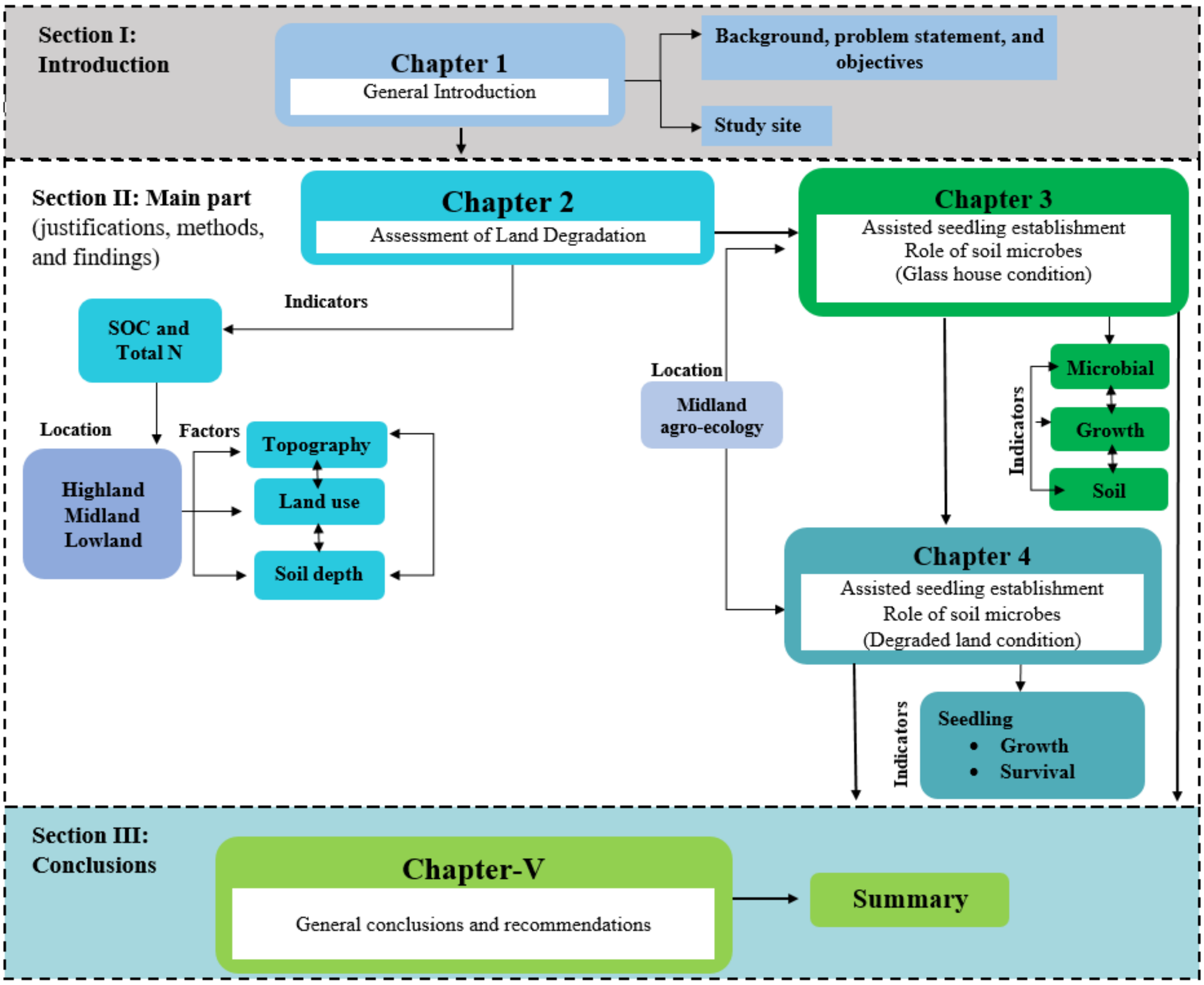

Figure 1-8. Structure of the thesis.

Chapter 2

Assessment of land degradation status using SOC and Total N stocks in different land use, topographic position and three agro-ecosystems

This chapter is mostly adopted from published as:

Abebe, G., Tsunekawa, A., Haregeweyn, N., Takeshi, T., Wondie, M., Adgo, E., Masunaga, T., Tsubo, M., Ebabu, K., Berihun, M. L. & Tassew, A. 2020. Effects of Land Use and Topographic Position on Soil Organic Carbon and Total Nitrogen Stocks in Different Agro-Ecosystems of the Upper Blue Nile Basin. Sustainability, 12, 2425.

2.1 Introduction

Soil organic carbon (SOC) and total nitrogen (TN) provide information on the impact of land management on soil health. The SOC stock, which is a key component and the largest carbon pool in terrestrial ecosystems, is strongly linked to nitrogen availability (Chen et al., 2016) and serves as an indicator of soil quality (Bünemann et al., 2018). SOC acts as a major source or sink for atmospheric CO2 (Lal, 2010; Martin et al., 2010; Zhang et al., 2014; Assefa et al., 2017b). Globally, soil is estimated to store 3150 Pg C (1 Pg C = 1015 g C), which is four times greater than carbon storage in the terrestrial plant biomass (650 Pg C) and atmospheric (750 Pg C) pools (Fan et al., 2016). The size of the soil carbon pool, however, is significantly controlled by the balance between the input and output of carbon in an ecosystem. Therefore, any change in the size of the SOC stock potentially affects elemental cycling, land productivity, atmospheric CO2 concentration, and thus global climate (Lützow et al., 2006; Poeplau et al., 2011; Fan et al., 2016).

The amount of SOC in a terrestrial ecosystem is influenced by natural and anthropogenic factors (Sun et al., 2015). Human-induced land-use change causes a particularly substantial loss of SOC (IPCC, 2013; Gelaw et al., 2014; Fan et al., 2016). Land-use change is associated with ecosystem carbon change (Fu et al., 2010) and drives negative impacts on climate and the environment. Numerous studies have shown that deforestation and land-use change results in land degradation and poorer soil quality (Yimer et al., 2007; Poeplau et al., 2011; Meshesha et al., 2014; Gelaw et al., 2015). In Ethiopia, the conversion of natural vegetation to croplands or plantations is increasing due to population pressure and socio-economic drivers. This has implications for biodiversity decline, land productivity, desertification, and SOC dynamics (Lemenih et al., 2004; Chen et al., 2012; Alem and Pavlis, 2014; Assefa et al., 2017b; Guteta and Abegaz, 2017).

According to Assefa et al. (2017b), conversion of natural forest to cropland in the northern highland of Ethiopia accounted for 50% to 87% of the observed SOC reduction. Likewise, Kassa et al. (2017) reported that conversion of forest and agroforestry to croplands caused an annual decline of SOC stock from 3.3 to 8.0 Mg ha^{-1} in the southwestern highlands of Ethiopia. On the other hand, reports on vegetation restoration of degraded lands in the region indicated that SOC is improved by planting *Eucalyptus* trees (Lemenih et al., 2004; Teferi et al., 2016; Feyisa et al., 2017) or establishing exclosures (Mekuria et al., 2017; Mekuria et al., 2018).

Generally, the soil of natural vegetation has higher SOC than croplands because of its higher organic residue content (Solomon et al., 2000). However, the efficiency of SOC accumulation depends on the quality and amount of organic inputs, decomposition rate in the soil (Solomon et al., 2000), and topographic position (Dessalegn et al., 2014; Sun et al., 2015). Topography influences SOC mainly by altering the input and output of carbon via hydrological processes, and it affects soil erosion and sediment deposition (Dialynas et al., 2016). The topographic position also affects water availability, temperature regime, vegetation distribution, and soil processes (Yimer et al., 2007; Wang et al., 2011).

Recently, owing to their strong influence on sustainability of natural and agricultural ecosystems, the effects of factors such as land use, topography, and their interaction on SOC and TN stocks have attracted scientific attention at the small watershed scale (Yimer et al., 2007; Fu et al., 2010; Dessalegn et al., 2014; Gelaw et al., 2014; Zhu et al., 2014; Sun et al., 2015). At the regional scale, climate is the dominant factor that controls SOC and TN stocks by inducing changes in soil moisture, vegetation patterns, decomposition rate (Conant et al., 2011), microbial activity (Crowther et al., 2015), and soil respiration (Wang and Fang, 2009). Therefore, SOC and TN dynamics in the soil vary in response to environmental factors (both biotic and abiotic) and are sensitive to changes in climate and the local environment (Chen et al., 2016). Thus, understanding soil carbon and nitrogen stock dynamics in different agro-ecosystems as a function of topographic position, land use, and their interaction is important for designing sustainable land management options (Dessalegn et al., 2014; Takoutsing et al., 2015; Teferi et al., 2016) that also contribute to food security (Lal, 2004).

The direct and interactive effects of topography and land use on SOC and TN stocks are not well studied in the landscape of Ethiopia's Upper Blue Nile basin, which is also known as the Abay River basin and covers an area of 173,000 km2 (Haregeweyn et al., 2017). The climate of the region is tropical highland monsoonal (Gebremicael et al., 2013). The region is characterized by fragile and drought-prone areas with diverse agro-ecosystems and severe land degradation. Although soil and water conservation practices have been used since the 1980s (Sultan et al., 2017), a reduction in vegetation cover (Sisay et al., 2017; Berihun et al., 2019) and soil erosion induced by poor land-use management have become major challenges for ensuring food security (Haregeweyn et al., 2017).

The aim of this study was to assess the effects of major controlling factors on SOC and TN stocks in three agro-ecosystems of the Upper Blue Nile basin. The specific objectives were to (1) determine how stocks of SOC and TN vary with topographic position, land-use type, and soil depth across agro-ecosystems; (2) assess the interactive effect of land use and topographic position on SOC and TN stocks within and across the three agro-ecosystems of the Upper Blue Nile basin.

2.2 Materials and methods

2.2.1 Study sites

The study was conducted in three different agro-ecosystems of the Upper Blue Nile basin, Ethiopia (Figure 2-1). Guder, Aba Gerima, and Dibatie represented the highland, midland, and lowland agro-ecosystems, respectively (Table 1-1). According to (Mekonnen, 2016), the four dominant soil types (in the FAO classification system) of the study area are Acrisols, Leptosols, Luvisols, and Vertisols (Table 1-1).

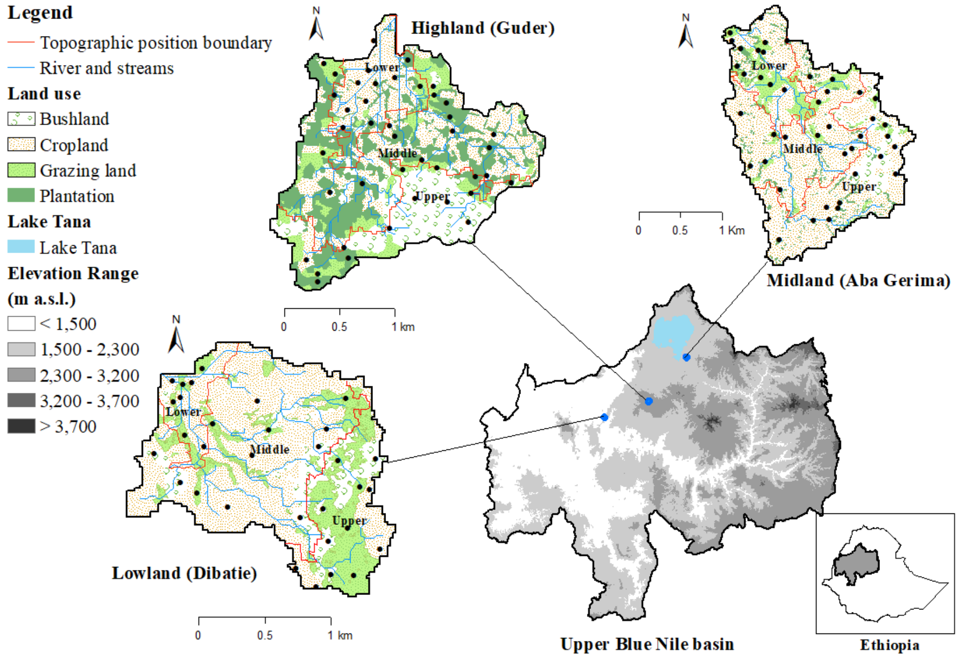

Figure 2-1. Location of the three study sites in the Upper Blue Nile basin, with respective land-use and drainage maps shown. The points in each watershed illustrate the distribution of sampling points with respect to land use and three topographic positions.

2.2.2 Soil sampling

Based on the available land-use types and elevation range of the watersheds (Table 1-1), three topographic positions (i.e., upper, middle, and lower) were selected. Cropland and grazing land are common in all topographic positions at the three study sites, whereas plantation (*A. decurrens* or *E. camaldulensis*) at Dibatie and bushland in the lower position at all sites are not part of the current land-use systems (Figure 2-1). In each topographic position, four replicated land uses were measured. A total of 352 soil samples were collected from the three agro-ecosystems. The top 50 cm of soil was sampled, divided into three soil layers of 0–15, 15–30, and 30–50 cm. Soil samples were collected from five points, at the four corners and in the center of a plot (10 m × 10 m) using a hand-driven soil auger. Soil samples collected from each plot from similar layers were thoroughly mixed to obtain a composite sample (1 kg). Soil bulk density was determined separately by using a metal core cylinder (100 cm^3), which was inserted at the midpoint of the 0–15, 15–30, and 30–50 cm layers. All composite soil samples were first air-dried and then passed through a 2-mm sieve, packed, labeled, and transported to Japan for chemical analysis at the Arid Land Research Center of Tottori University.

2.2.3 Soil analysis

Soil pH and electrical conductivity were measured at a 1:5 soil-to-water ratio using a pH meter (D-51, Horiba, Kyoto, Japan) and conductivity meter (ES-51, Horiba), respectively. Bulk soil density (Mg m^{-3}) was determined for core soil samples after oven-drying at 105 °C for 24.

2.2.4 Determination of SOC and TN stocks

Five-gram subsamples of homogenized soil from each soil depth were dried at 60 °C for 48 h. From each subsample, 1 g of soil was taken, and total organic carbon and nitrogen were determined using a CN corder (Macro Corder JM1000CN, J-Science Lab, Kyoto Japan). Total carbon and nitrogen stocks (Mg ha^{-1}) down to the 50 cm soil horizon were calculated using the model of (Ellert and Bettany, 1995):

$$\text{SOC (or TN) stock} = \text{content} \times \rho b \times d \times 10{,}000\ \text{m2}\ \text{ha}^{-1} \times 0.001\ \text{Mg kg}^{-1}$$

where SOC (or TN) stock is the soil organic carbon or total nitrogen stock (Mg ha^{-1}), content is the soil organic carbon or total nitrogen concentration (kg Mg^{-1}), ρb is the soil bulk density (Mg m^{-3}), and d is the thickness of the soil layer (m).

2.2.5 Data analysis

Data with a non-normal distribution were transformed using square-root and log transformation techniques. Two-way (within agro-ecosystem) and nested three-way (between agro-ecosystems) analysis of variance were used to test the significance of mean differences in SOC and TN content and stock as dependent variables, while topographic position, land use, soil depth, and their interactions (between two or three factors) were considered as driving factors. Differences in means between groups were analyzed using Tukey's HSD (honestly significant difference) test within the Agricolae package (version 1.2-8). Statistical analyses were carried out in RStudio (R Core Team, 2018), an interface for the R software program (version 3.4.4). The significance level was set at alpha = 0.05.

2.3 Results

2.3.1 Effect of topographic position on SOC and TN contents and stocks

At Guder, SOC content in croplands increased significantly ($p < 0.05$) from the upper (10.96 mg g−1) to the lower topographic position (16.68 mg g^{-1}; Figure 2-2a). In the case of grazing land, SOC content decreased from 22.59 mg g^{-1} in the upper position to 14.57 mg g−1 in the middle position and then increased to 17.40 mg g^{-1} in the lower position. For bushland and *A. decurrens* plantations, SOC content did not vary among topographic positions. TN content for bushland decreased significantly from 2.87 mg g^{-1} in the upper position to 2.39 mg g^{-1} in the middle position (Figure 2-2d). However, TN in cropland and grazing lands were not significantly different across topographic position.

At Aba Gerima, SOC and TN contents differed among topographic positions ($p < 0.05$; Figure 2-2b, e). The TN contents of bushland (0.78 mg g^{-1}), cropland (0.19 mg g^{-1}), and *Eucalyptus* plantations (0.49 mg g^{-1}) were significantly lower at the upper position than that at the middle and lower positions. The highest contents of SOC (17.52 mg g^{-1}) and TN (1.23 mg g^{-1}) were in bushland at the middle position, whereas croplands in the upper position showed the lowest SOC (4.78 mg g^{-1}) and TN contents (0.19 mg g^{-1}).

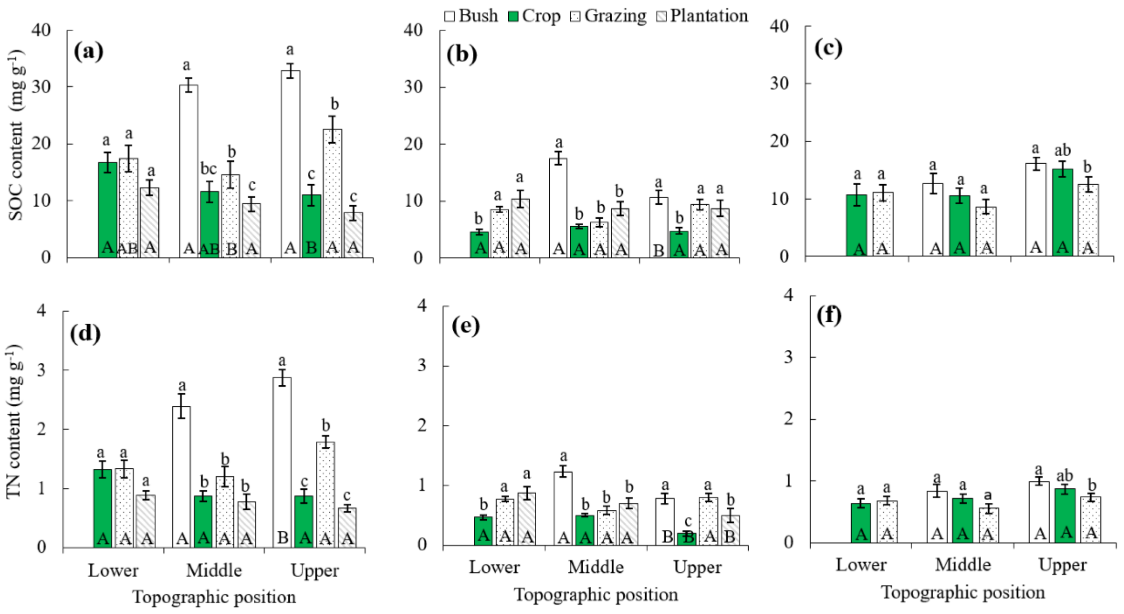

Figure 2-2. Soil organic carbon (SOC) and total nitrogen (TN) contents under different land uses and topographic positions at Guder (a, d), Aba Gerima (b, e), and Dibatie (c, f). Different lowercase letters above the bars indicate significant differences in SOC and TN among land uses in the same topographic position ($p < 0.05$); different capital letters indicate significant differences in SOC and TN among topographic positions within the same land use ($p < 0.05$). Error bars represent the standard error of the mean at alpha = 0.05

At Dibatie, SOC and TN contents in the upper position in bushland and cropland were 16.12 and 0.99, and 15.22 and 0.86 mg g^{-1} higher, respectively, than those in grazing land (12.58 and 0.74 mg g^{-1}; Figure 2-2c, f). In contrast, both SOC and TN contents were similar among land-use types in the middle and lower topographic positions.

At Guder, the SOC stock decreased from the upper to lower topographic positions in grazing land (Figure 2-3a). SOC stock under *A. decurrens* plantations increased significantly ($p < 0.05$), from 42.73 Mg ha^{-1} in the upper position to 44.63 Mg ha^{-1} in the middle position and 58.94 Mg ha^{-1} in the lower position. The SOC stock in bushland was highest (166.22 Mg ha^{-1}) in the upper position. The TN stock was significantly higher (13.11 Mg ha^{-1}) in grazing lands in the upper position and decreased toward the lower position (Figure 2-3d).

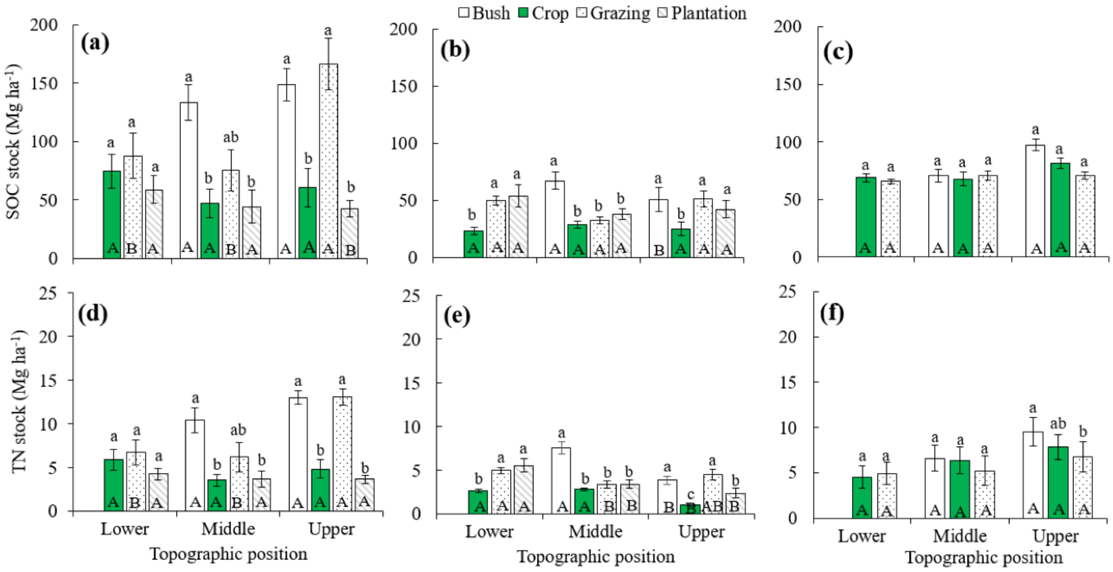

Figure 2-3. Soil organic carbon (SOC) and total nitrogen (TN) stocks under different land-use types and topographic positions at Guder (a, d), Aba Gerima (b, e), and Dibatie (c, f). Different lowercase letters above the bars indicate significant differences in SOC and TN among land-use types in the same topographic position ($p < 0.05$); different capital letters indicate significant differences in SOC and TN among topographic positions within the same land-use type ($p < 0.05$). Error bars represent the standard error of the mean at alpha = 0.05.

Table 2-1. Results of two-way analysis of variance (ANOVA) for SOC and TN contents and stocks as a function of topographic position and land use in different agro-ecosystems of the Upper Blue Nile basin.

Agro-ecosystem	Source	df	P-value			
			SOC content	TN content	SOC stock	TN stock
Guder	topographic position	2	0.278	0.093	< 0.001	< 0.001
	land use	3	< 0.001	< 0.001	< 0.001	< 0.001
	topographic position × land use	5	0.034	0.019	< 0.001	< 0.001
Aba Gerima	topographic position	2	0.562	< 0.001	0.307	< 0.001
	land use	3	< 0.001	< 0.001	< 0.001	< 0.001
	topographic position × land use	5	0.012	0.002	< 0.001	< 0.001
Dibatie	topographic position	2	0.010	0.003	0.046	0.005
	land use	2	0.024	0.003	0.492	0.004
	topographic position × land use	3	0.924	0.681	0.766	0.793

Notes: Topographic positions: upper, middle, and lower; land uses: bushland, cropland, grazing land, and plantation.

2.3.2 SOC and TN contents and stocks for different land uses across soil depths

Across soil profiles, both SOC and TN contents were slightly decreased from top to the lower soil profile at Aba Gerima (Figure 2-4b, e) compared with Guder (Figure 2-4a, d) and Dibatie (Figure 2-4c, f) The SOC and TN contents at Guder (Figure 2-4a, d) and Aba Gerima (Figure 2-4b, e) varied significantly among land uses at all soil depths ($p < 0.05$). At Dibatie, except for TN contents in the lower soil depth, there were no significant differences in SOC and TN contents among land-use types at all soil depths (Figure 2-4c, f).

At Guder, SOC and TN contents of bushland were significantly higher than the other land uses in all soil profiles (Figure 2-4a, d). At Aba Gerima, SOC contents in the 0–15 cm layer were 15, 11.3, and 6.7 times higher in bushland, plantation, and grazing land, respectively, than in cropland (Figure 2-4b). However, in the 15–30 and 30–50 cm layers, the SOC content in bushland was significantly greater than that in the other land uses. Similarly, TN content in the 0–15 cm layer at Aba Gerima was significantly higher ($p < 0.05$; 1.06 mg g−1) in bushland than in the other land uses (Figure 2-4e). In the 15–30 and 30–50 cm soil layers, the TN content was 1.29, 0.81, and 0.77 times higher in bushland, grazing land, and plantation, respectively, than in cropland ($p < 0.05$). At Dibatie, there were no significant differences in SOC content among land-use types at all soil depths (Figure 2-4c). However, TN contents in the 30-50 cm soil layer were significantly higher in bushland and cropland than in grazing land (0.49 mg g^{-1}; $p < 0.05$; Figure 2-4f).

On the other hand, the SOC and TN stocks at Guder and Aba Gerima varied significantly among land uses within each soil profile ($p < 0.05$; Table S1). Significant differences in SOC stocks across soil depths within each land-use type were observed at Aba Gerima and Dibatie, whereas TN stock only varied significantly at Dibatie (Table S1). SOC stocks of grazing land and plantations showed a 0.43- and 0.44-fold decrease from the top layer to the lower layer, respectively, at Aba Gerima. The SOC stock in cropland decreased significantly across soil depths at Dibatie. TN stock in the 0–15 cm layer was higher than at 15–30 and 30–50 cm soil depths at Dibatie, whereas no significant difference was found in the TN stock between the 15–30 and 30–50 cm layers.

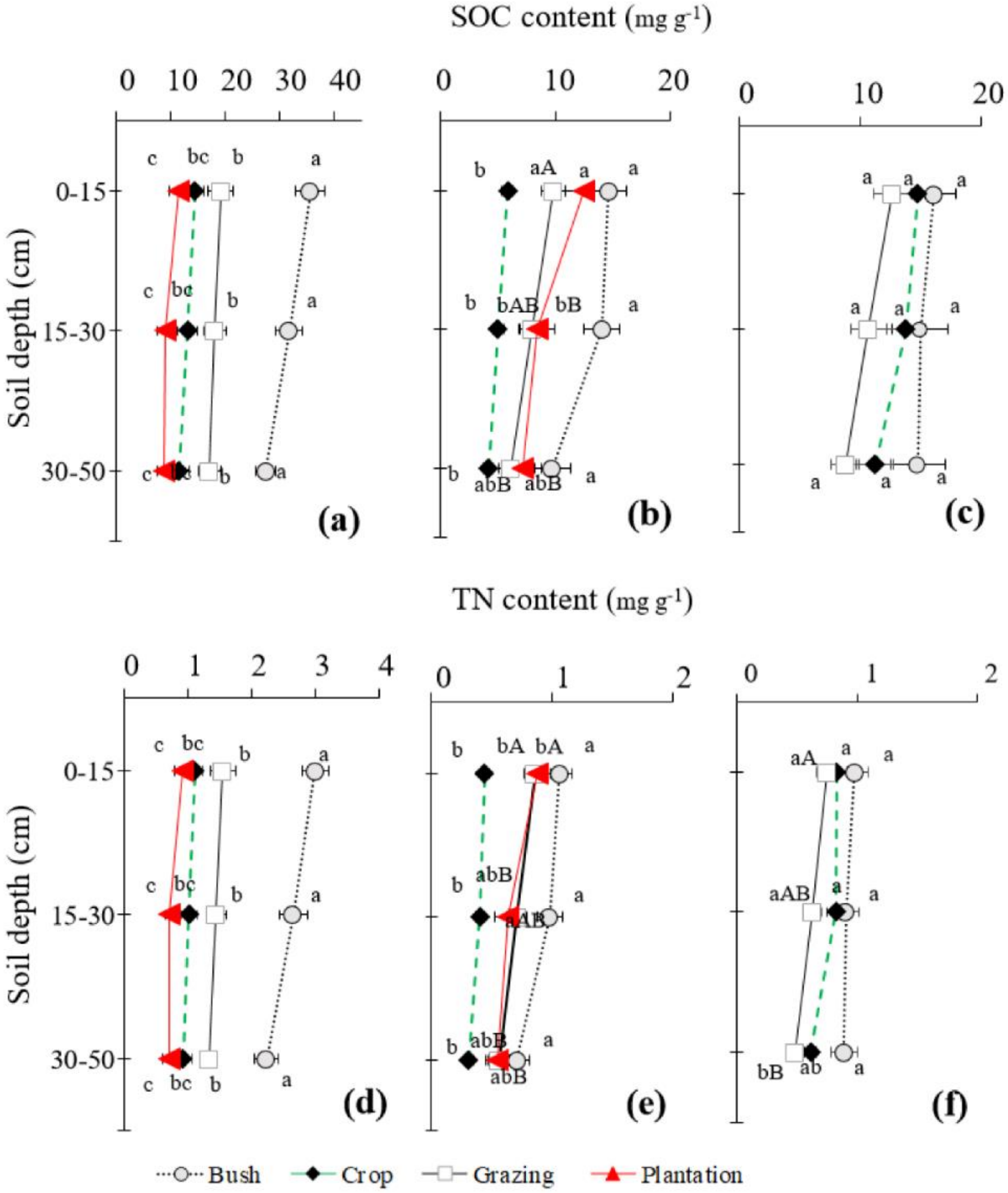

Figure 2-4. Soil organic carbon and total nitrogen contents in relation to land-use type at Guder (**a**, **d**), Aba Gerima (**b**, **e**), and Dibatie (**c**, **f**). Different lowercase letters indicate significant differences in SOC; n = 12.

Table 2-2. SOC and TN contents and stocks in different land-use types at the three study sites.

Land use	**Site**	**SOC**		**TN**		**C/N ratio**	**Bulk density**
		mg g^{-1}	**Mg ha^{-1}**	**mg g^{-1}**	**Mg ha^{-1}**		**Mg m^{-3}**
Bushland	Guder	31.63 (1.45) ^{a}	141.19 (6.74) ^{a}	2.63 (0.13) ^{a}	11.73 (0.50) ^{a}	12.16 (0.30) ^{a}	0.90 (0.01) ^{b}
	Aba Gerima	13.42 (1.16) ^{b}	59.23 (7.19) ^{b}	0.96 (0.09) ^{b}	4.13 (1.91) ^{b}	14.01 (0.31) ^{a}	1.09 (0.03) ^{a}
	Dibatie	15.31 (1.19) ^{b}	85.70 (9.81) ^{ab}	0.92 (0.06) ^{b}	8.08 (1.51) ^{a}	16.16 (0.57) ^{a}	1.11 (0.04) ^{a}
Cropland	Guder	13.07 (1.04) ^{a}	61.00 (2.33) ^{a}	1.02 (0.07) ^{a}	4.77 (0.28) ^{b}	12.52 (0.32) ^{b}	0.96 (0.03) ^{b}
	Aba Gerima	5.01 (0.29) ^{b}	25.97 (7.04) ^{b}	0.39 (0.03) ^{b}	2.14 (1.44) ^{c}	17.63 (2.33) ^{a}	1.12 (0.01) ^{a}
	Dibatie	13.28 (0.96) ^{a}	72.62 (8.33) ^{a}	0.75 (0.04) ^{a}	6.26 (1.34) ^{a}	17.38 (0.82) ^{a}	1.12 (0.03) ^{a}
Grazing	Guder	18.19 (1.20) ^{a}	109.94 (3.69) ^{a}	1.44 (0.09) ^{a}	8.68 (0.31) ^{a}	12.68 (0.20) ^{b}	1.18 (0.04) ^{b}
	Aba Gerima	7.97 (0.49) ^{b}	44.14 (7.55) ^{b}	0.71 (0.04) ^{b}	4.17 (1.19) ^{b}	11.03 (0.17) ^{b}	1.19 (0.03) ^{b}
	Dibatie	10.84 (0.81) ^{b}	66.42 (4.69) ^{b}	0.63 (0.05) ^{b}	5.65 (1.52) ^{ab}	17.52 (0.72) ^{a}	1.32 (0.02) ^{a}
Plantation	Guder	9.85 (0.87) ^{a}	48.79 (7.15) ^{a}	0.78 (0.06) ^{a}	3.86 (0.65) ^{a}	12.16 (0.41) ^{b}	1.02 (0.02) ^{a}
	Aba Gerima	9.24 (0.79) ^{a}	44.77 (6.33) ^{a}	0.68 (0.06) ^{a}	3.56 (0.38) ^{a}	17.15 (2.62) ^{a}	1.06 (0.03) ^{a}

Mean (standard error) values were calculated across the whole soil depth from 0 to 50 cm. Within a column, different letters for each land use indicate a significant difference between sites (Tukey's HSD at $P < 0.05$)

2.3.3 Effect of agro-ecosystem on SOC and TN contents and stocks

SOC and TN contents and stocks in bushland and grazing land at Guder were significantly larger than those at Dibatie and Aba Gerima (Table 2-2). SOC content in croplands at Aba Gerima (5.01 mg g^{-1}) was significantly lower than that at Guder (13.07 mg g^{-1}) and Dibatie (13.28 mg g^{-1}). Plantation at Guder (*A. decurrens*) and Aba Gerima (*Eucalyptus*) had similar SOC and TN stocks (Table 2-2). In contrast, the TN stock in cropland was significantly higher at Dibatie (6.26 Mg ha^{-1}) than at Guder (4.77 Mg ha^{-1}) and Aba Gerima (2.14 Mg ha^{-1}).

Both SOC and TN contents in the lower and middle topographic positions were significantly higher at Guder than at Aba Gerima and Dibatie (Table S2), whereas the SOC and TN contents in the upper position were significantly lower at Aba Gerima than at Guder and Dibatie. The SOC stocks in the upper position were in the following order: Guder (104.67 Mg ha^{-1}), Dibatie (88.90 Mg ha^{-1}), Aba Gerima (41.91 Mg ha^{-1}). The SOC stock in the middle and lower positions and TN stock in all topographic positions were significantly lower at Aba Gerima, but the values for Guder and Dibatie were similar (Table S2).

There was significant variation in the C/N ratios of cropland, grazing land, and plantation among sites (Table 3). The highest (17.52) and lowest (11.03) C/N ratios were those of grazing lands at Dibatie and Aba Gerima, respectively. *Eucalyptus* plantations (12.15) showed a significantly higher C/N ratio than *A. decurrens* plantations (12.16). The C/N ratios in the upper position were significantly higher at Aba Gerima followed by Dibatie and Guder, whereas the C/N ratios in the middle and lower positions were significantly higher at Dibatie than at the other two sites (Table S2).

Bulk densities in bushland, cropland, and grazing land differed significantly among sites ($p < 0.05$; Table 3), whereas those of plantations at Guder (*A. decurrens*) and Aba Gerima (*Eucalyptus*) were not significantly different. Bulk density ranged from 0.90 to 1.18 Mg m^{-3}, from 1.09 to 1.19 Mg m^{-3}, and from 1.11 to 1.32 Mg m^{-3} at Guder, Aba Gerima, and Dibatie, respectively (Table 3). Bulk densities in bushland, cropland, and grazing land were significantly lower at Guder than at Aba Gerima and Dibatie (Table 2-2). The bulk density also varied significantly among topographic positions ($p < 0.05$; Table S2). Soils at Guder showed significantly lower soil bulk density in the lower and middle positions as compared to the upper position. Soils at Dibatie had significantly higher soil bulk density in the middle position (Table S2).

Overall, SOC and TN contents and stocks were strongly dependent on agroecosystem ($p < 0.05$), land use ($p < 0.05$), and the interaction between agroecosystem and land use ($p < 0.05$; Table 4). Topographic position ($p < 0.05$) also influenced SOC content, SOC stock, and TN stock, but not TN content. In addition, the interaction between agroecosystem and topographic position affected TN content and SOC and TN stocks. TN content, SOC stock, and TN stock were also strongly dependent on the interaction of agroecosystem, topographic position, and land use.

Table 2-3. Nested three-way ANOVA for SOC and TN contents and stocks as functions of topographic position and land use across agroecosystems.

Source	**df**	**P-value**			
		SOC Content	**TN Content**	**SOC Stock**	**TN Stock**
Agroecosystem	2	< 0.001	< 0.001	< 0.001	< 0.001
Topographic position	2	0.043	0.862	0.019	< 0.001
Land use	3	< 0.001	< 0.001	< 0.001	< 0.001
Agroecosystem × topographic position	4	0.082	< 0.001	0.003	< 0.001
Agro-ecosystem × land use	5	< 0.001	< 0.001	< 0.001	< 0.001
Agroecosystem × topographic position × land use	13	0.058	< 0.001	< 0.001	< 0.001

Agroecosystems: Guder, Aba Gerima, and Dibatie; topographic positions: upper, middle, and lower; land uses: bushland, cropland, grazing land, and plantation.

2.4 Discussion

2.4.1 Effects of land-use type on SOC and TN contents and stocks across topographic positions

Climate, soil type, land use, and topography are the principal factors that control SOC and TN distributions at a regional scale (Li et al., 2012; Wiesmeier et al., 2019). In a small watershed, however, soil type and climate variability are commonly low (Chai et al., 2015). Our findings confirmed that land use and topography influenced the SOC and TN storage in the three agroecosystems.

At Guder, the SOC content in cropland was significantly increased (Figure 2-2a) from the upper to lower topographic position. In fact, the upper position of a watershed is often exposed to soil erosion, serving as a source of runoff and sediment for the lower positions (Sun et al., 2015). In cropland particularly, this situation has amplified the variation of SOC content in association with geomorphologic processes. Cropland in the highlands is poor in vegetation cover and experiences soil disturbance due to tillage and high biomass removal (Teferi et al., 2016; Assefa et al., 2017b). In contrast, SOC content in grazing land was significantly decreased from the upper to lower position (Figure 2-2a). Less soil disturbance, greater vegetation cover, and organic input from grazing animals would improve the SOC in the upper position. Similarly, Mekuria et al. (2018) reported better vegetation cover and biomass in communal grazing lands in the upper position than in the lower position, which is more easily accessed by livestock that induce changes in SOC. (2014) also found an increasing trend in SOC content for cropland and a decreasing trend in grassland from the summit to the lower part of a watershed in China. A review by (Deng et al., 2016) of studies conducted worldwide revealed that conversion of native vegetation to grassland significantly increased the SOC stock. In contrast, the SOC content in bushland and plantation at Guder were not affected by topographic position. This distribution pattern may be due to the generally good vegetation cover in bushland and plantation, which may reduce soil erosion in the upper position, resulting in similar SOC contents in the middle and lower positions. Likewise, (Fu et al., 2010) reported uniform SOC contents under different vegetative types along a hillslope on the Loess Plateau of China.

The TN content in bushland at Guder was higher in the upper than middle position, which was probably due to the presence of a large number of native leguminous shrubs (e.g., *E. abyssinica*) and trees (e.g., *A. abyssinica* and *A. gummifera*) in the bushland. These results corresponds with the finding of (Kassa et al., 2017), who reported high TN content in native vegetation consisting of leguminous tree species. TN stock of the grazing land was significantly higher (13.11 Mg ha^{-1}) in the upper position than in the middle and lower positions (Figure 2-3d), likely because grazing land in the upper position was recently converted from bushland (Berihun et al., 2019) which may have stored relatively high SOC and TN stocks.

At Aba Gerima, both SOC and TN contents were significantly different among land uses

(Figure 2-2b, e). SOC and TN contents in the middle position of bushland (17.52 and 1.23 mg g^{-1}, respectively) were higher than those in the upper position. Similarly, a study conducted in northern Ethiopia (Berihu et al., 2016) reported higher SOC and TN contents in the middle position of natural vegetation. Our results could be associated with soil erosion, which is a common problem in the study area and elsewhere in Ethiopia (Haregeweyn et al., 2017; Ebabu et al., 2019). Soil erosion often causes translocation of soil from the upper slope to lower area and contributes to the loss of soil organic matter (Yimer et al., 2007). Many studies elsewhere in the world (Zhu et al., 2014; Berihu et al., 2016; Ma et al., 2016) have reported that soil in sites of deposition has higher SOC and TN stocks.

At Dibatie, both SOC and TN contents in the upper position were significantly affected by land-use type (Figure 2-2c, f), which could be due to greater anthropogenic pressures in the upper than in the middle and lower topographic positions. Many members of the farming community live around the lower part of the watershed, and their livelihoods depend on the bushland. This results in continuous removal of wood and bushland clearing for cropland and grazing land toward the upper position. SOC contents in bushland and cropland were 1.41 and 1.22 times higher and TN contents were 1.46 and 1.19 times higher than those of grazing lands. Natural vegetation at Dibatie is dominated by deciduous tree and shrub species that commonly contribute large amounts of organic matter to the soil. However, grassland is regularly burned, which substantially reduces the grass cover and induces loss of SOC and TN contents (Knicker, 2007). In a study in Ethiopia, (van Breugel et al., 2015) reported that the natural vegetation in Dibatie (Combretum–Terminalia) decreased as a result of fire.

With regard to land-use effects, the SOC stock of grazing land soil decreased significantly from the upper (162.22 Mg ha^{-1}) to middle position (75.50 Mg ha^{-1}) at Guder. Soil bulk density in grazing lands is relatively higher as a result of livestock trampling (Don et al., 2011; Teferi et al., 2016; Mekuria et al., 2017). At this site, a bulk density of 1.18 Mg/m3 was recorded in the grazing land (Table 2-2). At Aba Gerima, a high SOC stock was stored in the middle position of bushland. Similarly, at Dibatie bushlands showed higher SOC and TN contents than those of the other land-use types.

2.4.2 Effect of soil depth on SOC and TN contents and stocks

At Guder, SOC and TN contents at 0–15 cm soil depth in bushland were higher than those of other land-use types (Figure 2-4a, d). Bushland comprises a sizable proportion of native vegetation, and the bushes, shrubs, and trees contain a substantial amount of wood biomass with a lower decomposition rate, which could improve the organic input and contribute more to soil SOC and TN. These results are similar to those of previous studies (Sun et al., 2015; Kassa et al., 2017) that reported higher SOC and TN contents in the surface soil under native vegetation as compared to that of other land uses. Therefore, conversion of bushland to another land-use type may cause a substantial amount of SOC loss from the surface soil, as reported by studies conducted elsewhere (Martin et al., 2010; Zhang et al., 2014; Kassa et al., 2017).

Plantation (*A. decurrens* woodlot) contained lower surface SOC and TN contents than we expected (Figure 6a, d). (Tesfaye et al., 2015) reported lower SOC and TN contents in *A. decurrens* plantation in the central highlands of Ethiopia, which reflects the complete removal of plant residues from the woodlots. The plantations were established on previous cropland areas, but due to prolonged soil disturbance and soil erosion this land was no longer able to support crop production. Thus, farmers had to change the cropland to plantation as a result of poor soil fertility and degradation (Nigussie et al., 2016; Wondie and Mekuria, 2018). Because plantations are commonly used for charcoal production, both the above- and belowground biomass is completely removed at the end of a rotation cycle (~3–5 years). According to Sultan et al. (2018), plantations have high stand density (< 1 m spacing), no understory vegetation cover, poor infiltration, and high runoff, all of which could contribute to their lower SOC and TN contents.

Similarly, in the 0–15 cm layer of Aba Gerima, cropland has significantly less surface SOC and TN contents than bushland, plantation, and grazing land (Figure 2-4b, e). This difference may be due to croplands having less organic input than areas with more vegetation. However, plantations at Aba Gerima had a SOC contents comparable to those of cropland and grazing lands. These differences in SOC content from the plantations at Guder are likely induced by the difference in species and woodlot management. Unlike the Acacia plantations at Guder, the plantations at Aba Gerima consist of *Eucalyptus camaldulensis* and tree harvesting operations do not include the belowground biomass. A study in northern Ethiopia revealed that *Eucalyptus* plantations had a better potential to restore SOC content than did cropland and grazing land (Teferi et al., 2016; Assefa et al., 2017b), and Tesfaye et al. (2016) reported that the conversion of cropland to *Eucalyptus* plantations ameliorates soil degradation in central Ethiopia. Moreover, Assefa et al. (2017a) reported that the amount of fine root biomass in *Eucalyptus* plantations was higher than that of cropland and grazing land.

In the lower soil depths (15–30 and 30–50 cm) at Guder, SOC and TN contents were similar to those of the surface layer, probably largely due to plant roots and exudates, dissolved organic matter, bioturbation, and translocation of particulate organic matter (Twongyirwe et al., 2013). This result is in line with the finding of (Tesfaye et al., 2016), who reported a similar trend across soil depths. At Dibatie, soil depth generally had no effect on SOC content, but in the lower depths TN content was higher in bushland than in grazing land. This may be because of regular burning of the surface cover in woodland, which is the most common soil fertility problem in the lowlands of northwestern Ethiopia (Lemenih et al., 2007), as well as leaching and lower temperature in the subsurface layer (Knicker, 2007).

Land use had a significant effect on both SOC and TN stocks across the entire 50 cm soil profile at Guder (Table S1). The topsoil layer of bushland stored significantly greater SOC and TN stocks than that of plantation, which may be largely due to less carbon input from litter biomass, roots, and residues including understory biomass in plantations (Stockmann et al., 2013; Gelaw et al., 2014; Sun et al., 2015; Wang et al., 2016). At the lower two depths, however, bushland and grazing land had the highest SOC and TN stocks. Similarly, at Aba Gerima SOC and TN stocks of cropland were lower than those of the tree- and grass-based systems of bushland, plantation, and grazing land. Many studies have reported that cropland stores the lowest SOC and TN stocks (Dessalegn et al., 2014; Deng et al., 2016). In the 30–50 cm soil layer, bushland also showed higher SOC and TN accumulation than cropland (Table S2). At Dibatie, however, SOC and TN stocks were similar at all soil depths, except for the TN stock in the lower soil depth. This could be due to the practice of burning woodland (as discussed above).

2.4.3 Effect of agro-ecosystem on SOC and TN contents and stocks

Agro-ecology had a significant effect on SOC and TN contents and stocks (Table 2-3). The soil under bushland and grazing land had lower SOC and TN contents at Aba Gerima and Dibatie than at Guder. In different ecosystems, climate strongly affects the soil carbon and nitrogen by controlling vegetation productivity and organic matter decomposition

(Deng et al., 2014). Guder had higher mean annual precipitation and was cooler than the other two sites (Figure 1-7). Similar studies also reported that areas with high mean annual precipitation and lower mean annual temperature tend to accumulate large amounts of SOC and TN (Li et al., 2012; Deng et al., 2014; Assefa et al., 2017b). The SOC and TN contents in cropland were less at Aba Gerima than at Guder and Dibatie. This result clearly indicated that cropland at Aba Gerima had less organic input and poor physical protection including vegetation cover, which plays a substantial role in organic matter stabilization in cultivated land (Liu et al., 2016). In another study of agro-ecosystems of the Upper Blue Nile basin, (Ebabu et al., 2019) reported greater soil loss for cropland at Aba Gerima than that at Guder and Dibatie. However, plantations had similar SOC and TN contents at Guder and Aba Gerima.

The SOC stock in bushland was larger (141.19 Mg ha^{-1}) and grazing land (109.94 Mg ha^{-1}) at Guder than at Aba Gerima. These values are comparable with previous reports of SOC stocks of 69–239 and 67–109 Mg ha^{-1} in natural vegetation and grazing land to 50 cm depth in the northwest highlands of Ethiopia (Tesfaye et al., 2016; Assefa et al., 2017b), but they are markedly higher than the values reported by (Gelaw et al., 2014), who recorded SOC stocks of 52 and 39 Mg ha^{-1} to 50 cm depth in grazing and shrub land of northern Ethiopia, respectively. These values are also lower than the estimated mean of tropical sites (216 Mg ha^{-1}; (Lal, 2004)) and the global average (254 Mg ha^{-1}; (Batjes, 1996)). However, cropland had higher SOC and TN stocks at Dibatie than those at Guder and Aba Gerima, which could be related to the different farming system at Dibatie. Unlike at Guder and Aba Gerima, crop residues are not collected in the field at Dibatie, which could be contributing to the SOC accumulation. In addition, the bulk density in cropland at Dibatie was higher than that at the other sites (Table 2-2). On the other hand, cropland at Dibatie is a new land-use type, having been converted from woodlands (Combretum–Terminalia) recently. In southern Ethiopia, (Tesfaye and Negash, 2018) reported that soil under Combretum–Terminalia vegetation stored higher carbon stock than the aboveground biomass.

2.4.4 Implications of SOC and TN stocks as indicators for sustainable land management in the Upper Blue Nile basin

At the watershed scale, the effects of topographic position and land use on SOC and TN stocks were not consistent. At Guder, Aba Gerima, and Dibatie, topographic position and land use, land use, and topographic position, respectively, were the dominant factors that affected SOC stock (Table 2). However, TN stock in all agro-ecosystems was affected by topographic position and land use. Thus, by maintaining the same land uses at Guder, both stocks of SOC and TN could be enhanced by topographic position, whereas converting bushland and grazing land to *A. decurrens* woodlots would likely diminish the SOC and TN stored in the soil. At Aba Gerima, conversion of cropland to *Eucalyptus* plantation had a positive impact on SOC and TN (Tesfaye et al., 2016; Assefa et al., 2017b). Plantation had lower SOC and TN stocks due to poor undergrowth and litter removal (Temesgen et al., 2016). The interaction of land use and topographic position showed a significant effect on SOC and TN stocks at Guder and Aba Gerima (Table 2), indicating that the variation in topography and land use may simultaneously affect different soil processes including soil erosion and the accumulation and decomposition of organic matter (Zhu et al., 2014; Sun et al., 2015).

Across the agro-ecosystems, topographic position and land use were the main factors influencing SOC and TN stocks, but agro-ecosystem also showed a significant interactive effect with topographic position and land use on the SOC and TN stocks (Table 6). Among agro-ecosystems, the spatial distributions of SOC and TN stocks were higher at Guder followed by Dibatie and Aba Gerima (Figure 2-3). In addition to vegetation composition, the hydrological regime, soil formation processes, and climate (temperature and precipitation) are important factors that affect the SOC spatial distribution (Deng et al., 2014), which in turn influences soil respiration (Wang and Fang, 2009). In this study, Guder has higher mean annual precipitation and lower mean annual temperature (Figure 2a) than Aba Gerima (Figure 2b) and Dibatie (Figure 2c). Agro-ecosystems in cooler and moister climates accumulate high SOC and have a low rate of soil respiration (Wang and Fang, 2009) and limited microbial activity (Wiesmeier et al., 2019). A warm and moist agro-ecosystem like Dibatie, however, tends to store moderate SOC stocks due to high biomass production (Table 1) and greater soil respiration. Aba Gerima has low SOC and TN stocks, likely as a result of severe soil erosion, prolonged crop cultivation, and poor land management. The C/N ratio varied from 11–13 at Guder to 10–21 at Aba Gerima and 15–18 at Dibatie. The C/N ratio is commonly considered as an indicator of microbial activity and quality of soil organic matter (Ostrowska and Porębska, 2015). Like the SOC and TN stocks, the C/N ratio also varied among land-use types**,** agro-ecosystems, and topographic positions.

2.5 Conclusions

This study clearly demonstrated that SOC and TN stocks varied significantly across land-use types and topographic positions of different agro-ecosystems. Poor and environmentally damaging land management practices tended to reduce SOC and TN in soil. Interactive effects of topographic position and land-use types on SOC and TN stocks were significant at Guder and Aba Gerima. Bushland at Guder accumulated a substantial amount of SOC and TN stocks. Cropland at Aba Gerima had poor SOC and TN stocks. Compared to other land-use types, the soil of *A. decurrens* plantation was the lowest in SOC and TN due to high biomass removal and improper silvicultural management. However, *E. camaldulensis* plantations at Aba Gerima had a positive impact on SOC and TN stocks. Across agro-ecosystems, Guder and Dibatie accumulated larger SOC and TN stocks than those of Aba Gerima.

Overall, land use was a crucial factor influencing SOC and TN both within and across the sites. However, the effect of topographic position was more pronounced across watersheds than within them. Aba Gerima showed lower SOC and TN stocks due to prolonged crop cultivation and mismanagement of the landscape. This calls for immediate land management interventions particularly targeting croplands. Our findings highlight the importance of assessing SOC and TN stocks when designing evidence-based land management options in the Upper Blue Nile basin.

Chapter 3

Role of soil microbes in assisting seedling establishment under glasshouse condition

This chapter is mostly adopted from published as:

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3.1 Introduction

Land degradation is a major global problem affecting all terrestrial biomes in arid and humid regions (Nkonya et al., 2016; Mekuria et al., 2018). Human activities, such as deforestation, overgrazing, and improper agricultural practices are the main factors causing land degradation, all of which significantly reduce environmental quality, and socio-economic sustainability (Nunes et al., 2012; Araujo et al., 2014; Meshesha et al., 2014; Singh, 2015). Land degradation also causes deterioration of soil communities and negatively influences ecosystem function (Zhang et al., 2006). Studies have revealed the significant role of soil microbes in ecosystem functioning (van der Heijden and Horton, 2009; Neuenkamp et al., 2019). However, the loss of beneficial microbes, including fungi and bacteria, adversely affects the recovery potential of a degraded ecosystem (Rashid et al., 2016).

Symbiotic relationships between the roots of higher plant and microbes (fungi and bacteria) strongly influence plant survival, growth, and ecosystem properties (Classen et al., 2015). These beneficial microbes can enhance soil nutrient supply , drought tolerance , and pathogen resistance (Wehner et al., 2010) of the host plant. The interaction of soil microbes with plant roots and organic matter can improve soil aeration and resistance to slaking and erosion by enhancing soil aggregation and structural stability; the microorganisms influence soil aggregation via chemical stabilization, and the organic matter contributes a cementing effect (Rillig et al., 2015). However, these functional roles of soil microbes are limited in degraded ecosystems because of a low level of microbial diversity, poor vegetation cover, high soil disturbance, and severe erosion rates (Ambrosini et al., 2015; Rashid et al., 2016; Calderon et al., 2017). Consequently, in many regions of the world, various methods of ecological restoration are necessary to rectify degraded ecosystems (SER, 2004). For instance, soil and water conservation practices (Sultan et al., 2018), including afforestation and exclosure establishment (Damene et al., 2013; Mekuria et al., 2018), have been experimented within Ethiopia. However, achieving restored ecosystem function through re-establishment of native tree species (Aerts et al., 2007) has proved challenging due to the lack of a native soil microbial community in degraded lands (DL) (Delelegn et al., 2018). Indeed, soil microbes in combination with plant species play a crucial role in restoring DL (Ambrosini et al., 2015; Neuenkamp et al., 2019). In the case of the Ethiopian highlands, very little information exists about the source of native inoculum for the successful restoration of lost microbial community functions in degraded landscapes.

Small patches of natural forest, called "Church Forests," exist around Ethiopian Orthodox Tewahedo churches and monasteries, and these constitute the last remnants of the original forest cover, having been conserved for more than a century. Church Forests are located in a matrix of intensively degraded agricultural landscape (Wassie et al., 2010; Abiyu et al., 2016). Apart from their social and spiritual value, Church Forests are obvious and important foci of biodiversity and act as a source of seeds and germplasm for native flora (Wassie et al., 2010; Berhane et al., 2013; Abiyu et al., 2016; Aerts et al., 2016). However, there is a lack of studies characterizing the role of microbial communities or evaluating the inoculum potential and the effects of microbes from Church Forests on the early stages of native tree establishment.

Olea europaea L. subsp. cuspidata and *Albizia gummifera* are among the most important native tree species of Ethiopia. These two tree species were selected for this study based on their social and ecological importance and their limited survival and regeneration ability in degraded lands of the Ethiopian highlands (Wassie et al., 2009b; Wassie et al., 2009a). *Olea europaea* subsp. cuspidata (Wall. ex DC.) is a late-successional evergreen tree species found in dry Afromontane forest between 1250 and 3100 m a.s.l.(Friis, 1992) . The species is hardy and drought resistant once established, even in poor soils; adult trees are commonly 15-25 m high (Tesemma, 2007). *Albizia gummifera* (J.F.Gmel.) C.A.Sm., is a deciduous tree species; It can reach 15 m in height and occurs in the semi-humid and humid highland forest between 1400 and 2500 m a.s.l. (Tesemma, 2007). It often co-exists with *Olea europaea* and Juniperus procera (Aynekulu et al., 2011). Despite *Olea europaea* and *Albizia gummifera* are among the suitable native tree species supposed to restoring degraded lands in the highlands of Ethiopia, no or very limited information is available on growth performance and survival rate of their seedlings in soils from conserved forest and degraded lands. This study was, therefore, designed to: (1) assess the soil microbial diversity in remnant Church Forest and surrounding degraded land, (2) evaluate the effect of soil microbiome, from under the canopy of Church Forest, on early growth and survival rate of seedlings of *Olea europaea* and *Albizia gummifera* under glasshouse conditions, and (3) evaluate the association between soil microbial and chemical properties in relation to plant growth.

3.2 Materials and methods

3.2.1 Site description

The study was conducted using soil sampled from Ethiopian Orthodox Tewahedo Church, Laguna St. Giorgis forest (Figure 3-1). Laguna St. Giorgis forest is a remnant forest around a church built in 1500 A.D. It is located at 11°39′21″N and 37°30′36″E at an altitude of 2100 m a.s.l. The current forest covers 5.25 ha. The mean annual rainfall ranges from 895 to 2037 mm (Figure 3-2a). The mean annual temperature range is 17 °C–31 °C (Yibeltal et al., 2019a). The vegetation type of the area is *Albizia–Juniperus- Croton* -

dominated dry Afromontane forest (Cardelús et al., 2013). Leptosols and Regosols are the major soil types in the study area (Mekonnen, 2016).

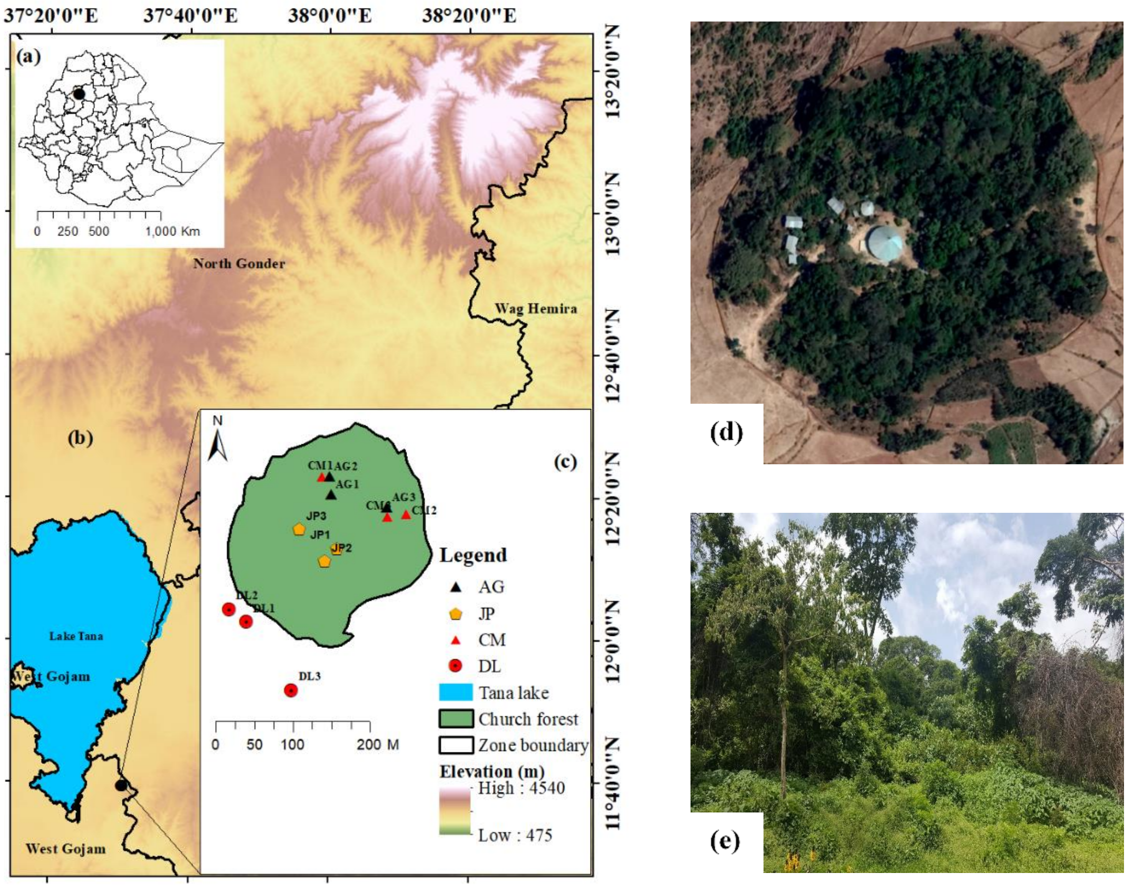

Figure 3-1. Location of the study area. The study site is indicated by the black circle (a, b). *Albizia* gummifera (AG), *Croton macrostachyus* (CM), *Juniperus procera* (JP) are tree species in the Church Forest, and DL is adjacent degraded land (c). Image of Aba Gerima Church Forest top view (d) and side view (e).

3.2.2 Field soil sampling

Soil samples were collected under the canopy of three predominant native tree species: *Albizia gummifera* (AG), *Croton macrostachyus* (CM), and *Juniperus procera* (JP), in the Church Forest (Figure 1c), and from adjacent degraded farmland (DL). For each tree species and DL, three replicate soil samples were collected from 0 to 20 cm soil depth with a ruler and a hand shovel and around 3.0 kg of soil put in plastic bags. Soil samples from a similar source were mixed to obtain a composite inoculum. The samples were prepared at the soil laboratory of Bahir Dar University, Ethiopia, and transported to Japan for the experiment: half of the total samples for each soil origin were sterilized using gamma-ray (30–60 kGy; (McNamara et al., 2003)) to evaluate the effects of soil microbes. For each soil origin, 2 g sample was stored in at -80 °C in the refrigerator for molecular analysis to evaluate soil microbiome before the experiment.

3.2.3 Experimental design

A pot experiment was carried out in a glasshouse at Tottori University, Arid Land Research Center, Japan. The temperature in the glasshouse was in the range of 21 °C–25 °C with an average relative humidity of 65.7% (Figure 3-2b).

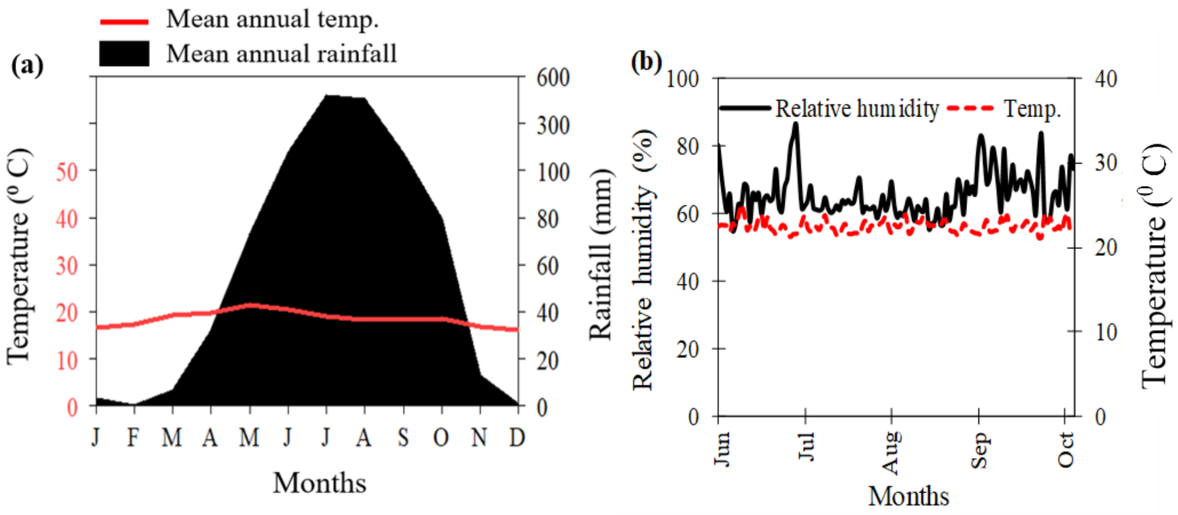

Figure 3-2. (a) Climate characteristics of the study area from 1962 to 2017 (Yibeltal et al., 2019a; Yibeltal et al., 2019b) and (**b**) glasshouse atmospheric conditions during the experiment. Climate diagram of the study site (**a**) is based on Walter and Lieth climate diagrams (Orlowsky, 2014).

Surface-sterilized seeds of *Olea* and *Albizia* were germinated, in May 2018, using autoclaved vermiculite (15 min at 121 °C) in a seed germination chamber (MLR-351H, SANYO, Tokyo, Japan); temperature and relative humidity of the chamber were 25°C and 60-70%, respectively. *Albizia* seeds were germinated in a week while *Olea* seeds took 2 weeks after sowing in the chamber; the seedlings were then transplanted in to glass pots (4 cm diameter and 14 cm height each) filled with sterilized and non-sterilized soil (100 g for samples from forest and 125 g for samples from degraded land) at the depth of 2-3 cm and kept for 2 weeks in a room condition (25 °C and 12 hours light).

A total of 128 pots (2 plant species × 4 soil origins × 2 treatments × 8 replicates) were transferred to the glasshouse for further monitoring and evaluation. Pots were maintained at 15% moisture content throughout the experiment period (5 months) and were weighed every 3 days. Every 2 weeks, 10 ml of sterilized distilled water was added to the non-sterilized pots and, to control contamination, 10 ml of 1:50 antibiotic (penicillin/streptomycin/amphotericin B solution (Orlowsky, 2014) and sterilized distilled water) solution was added to the sterilized pots.

3.2.4 Growth and survival data

Survival was recorded monthly until the end of the experiment. Plant height (cm) and root collar diameter (RCD, mm) were measured using a ruler and digital caliper, respectively. Root to shoot (R/S) ratio was calculated by dividing root dry weight by shoot dry weight.

3.2.5 Soil analysis

Soil samples collected from field and pots were air-dried, passed through a 2-mm sieve and analyzed for selected soil parameters: pH, organic carbon, total nitrogen, available phosphorus (P), aggregate–structure stability, and texture (particle size). Soil pH was measured in 1:2.5 soil: water suspension. Soil organic carbon (SOC) and total nitrogen (TN) were determined using a CN corder (Macro Corder JM1000CN, J-Science Lab, Kyoto Japan). Soil available P was extracted with a solution of 1.0 M ammonium fluoride and 2.5 M hydrochloric acid (Bray II method; Bray and Kurtz (1945)). Phosphate was detected by absorption spectrometry (UV–140–02; Shimadzu, Kyoto, Japan) using the molybdenum blue colorimetric procedure. Soil aggregate–structure stability (SAS) was determined using the modified high energy moisture characteristics method Bray and Kurtz (1945), and particle size was analyzed by the hydrometer method (Bouyoucos, 1936). In addition, soil moisture content was gravimetrically measured after drying moist samples in an oven at 105 °C for 24 h (Bouyoucos, 1936).

3.2.6 Plant and soil sample collection

Seedlings were harvested at 5 months after transplantation (Figure S2 and S3). At harvest, for each seedling: (i) the shoots (stem and leaves) were harvested and weighed, (ii) after breaking the glass pots, the roots were carefully separated, gently washed with tap water, and weighed in fresh. Shoots and roots were then dried at 60 °C for 72 h and weighed to estimate the shoot, root and total biomass. In each pot, 2 g of soil samples were collected and stored at -80 °C in the refrigerator for molecular analysis after the experiment.

3.2.7 Soil DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

For each sample, 0.25 g of soil was extracted using DNeasy PowerSoil® DNA kit (Qiagen, Germany) following the manufacturer's protocol. The extracted DNA solution was diluted 10 times and PCR was carried out using BIO-RAD T100™ Thermal Cycler (Bio-

Rad Laboratories, Hercules, CA). For each sample, two replicates were amplified in a 20 µL (total volume) reaction mixture, containing 1 µL of template DNA (10 ng/µL), 7.0 µL sterilized distilled water, 10 µL of 2× Q5 High-Fidelity DNA Polymerase (New England Biolabs Inc., MA, USA), and 1.0 µL for each primer. The V4 region in the 16S rRNA gene was amplified using primers S-D-Arch-0519-a-S-15 (CAGCMGCCGCGGTAA) and S-D-Bact-0785-a-A-21(GACTACHVGGGTATCTAATCC) for bacteria (Klindworth et al., 2013). The internal transcribed spacer (ITS) region was amplified with the primers ITS1F_KYO2 (TAGAGGAAGTAAAAGTCGTAA) and ITS2_KYO2 (TTYRCTRCGTTCTTCATC) for fungi (Toju et al., 2012).

Each forward primer was tagged with the Ion Torrent specific adapters and Ion Xpress barcode to distinguish the origin samples. The expected band size for 16S rRNA and ITS primers was 350 bp and 360 bp, respectively.

The PCR thermal cycling conditions were: initial denaturation at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 53 °C for 30 s, and 72 °C for 1 min, followed by a 2 min final extension at 72 °C. The PCR products were checked by agarose gel electrophoresis, and the replicates were composited to make one solution for purification. The purified DNA concentration was quantified for each PCR product with the Qubit dsDNA HS Assay Kit on a Qubit fluorometer 2.0 (Invitrogen, Carlsbad, CA, USA). Then, pooled DNA distribution and size were checked using Agilent 2100 (Agilent Technologies, Inc.) after putting an equimolar amount of amplified DNA into a tube. Following this, DNA sequencing by Ion Torrent Personal Genome Machine (Life Technology, Inc.) was performed as described by (Toju et al., 2012).

3.2.8 Sequence data processing

Sequence data processing was performed as previously described Tian et al. (2017).

Quality sequences were gained using the Quantitative Insight into Microbial Ecology (QIIME) 1.8.0 pipeline (Tedersoo et al., 2018) after removing sequences shorter than 200 bp for bacteria and 360 bp for fungi, and sequences with expected errors predicted by Phred (Q) scores greater than 0.8. ITSx were used to exactly pick the ITS region for fungal DNA (Caporaso et al., 2010). Successful sequences were clustered into operational taxonomic units (OTUs) at 97% similarity level using USEARCH (Bengtsson-Palme et al., 2013). Then, sequencing chimeras were checked and removed using UCHIME (Edgar, 2010). Taxonomy of each OTU was assigned to fungal and bacterial taxa using UNITE (Edgar et al., 2011) and SILVA (SILVA 128 QIIME release) databases, respectively.

3.2.9 Statistical analysis

Statistical analyses were performed in R (Team, 2016) using the interference implemented RStudio (version 1.1.383). The data were checked for normal distribution before analysis using the Shapiro–Wilk test, and non-normally distributed data were log and square-root transformed. The effects of soil from the four origins (DL, AG, CM, and JP) with microbial treatment on plant height, R/S ratio, mass, pH, soil organic carbon (SOC), total nitrogen (TN), carbon/nitrogen (C/N) ratio, available P, and SAS were analyzed with a general linear model procedure. Three-way ANOVA was used to check the effect of species on pooled plant and soil properties, then the two species (*Olea* and *Albizia*) separately and soil origin (with and without microbial treatment) were fixed factors. Two-way ANOVA was used to test the interaction between soil origin and microbial treatment on plant growth indices and soil properties. Differences in means across soil origin and microbial treatments were analyzed with Tukey's HSD test using the R package Agricolae (de Mendiburu, 2019). Survival was measured using the Kaplan–

Meier procedure; survival curves were compared statistically using log-rank test (LogranKA package) and Cox-regression (survival package) survival analysis was used to test the interaction between soil origin and microbial treatments (Allignol and Latouche, 2019).

Rarefied OTU tables were used to analyze soil microbial community composition and diversity Indices (Shannon (H′) and Simpson (D)) and number of OTUs observed. The effects of soil origin between treatments on H′, D, and number of OTUs observed were non-parametrically determined (Kruskal–Wallis test). To test the effect of soil origins and microbial treatments on microbial communities, PerMANOVA (1000 permutations) was performed by the Adonis function in the vegan package of R (Oksanen et al., 2018). Non-metric multidimensional scaling based on Bray–Curtis dissimilarity was used to visualize the results, and the relationships between soil properties, and microbial communities were tested using the envfit function in the vegan package of R (Oksanen et al., 2018).

3.3 Results

3.3.1 Soil microbial community

3.3.1.1 Soil bacterial community composition, abundance, and relationship with soil properties

Acidobacteria, *Actinobacteria*, *Crenarchaeota*, *Nitrospirae* and *Proteobacteria* were the most abundant bacterial phyla in the original forest soil (Figure 3a). Whereas, *Actinobacteria*, *Gammatimonadetes*, and *Proteobacteria*, were the most dominant bacterial phyla in the soil after plants (Figure 3-3a, c). For both plant species, the relative abundance of *Proteobacteria* was higher in sterilized soil (47% and 53%, respectively) than in non-sterilized one (28% and 29%, respectively), in contrast to the relative abundance of *Actinobacteria* was higher in non-sterilized soil. Also, in non-sterilized soil, the abundance of *Acidobacteria* was higher in DL, CM, and JP for *Olea* seedlings (Figure 3-3a) and in all soil origins for *Albizia* seedlings (Figure 3-3c).

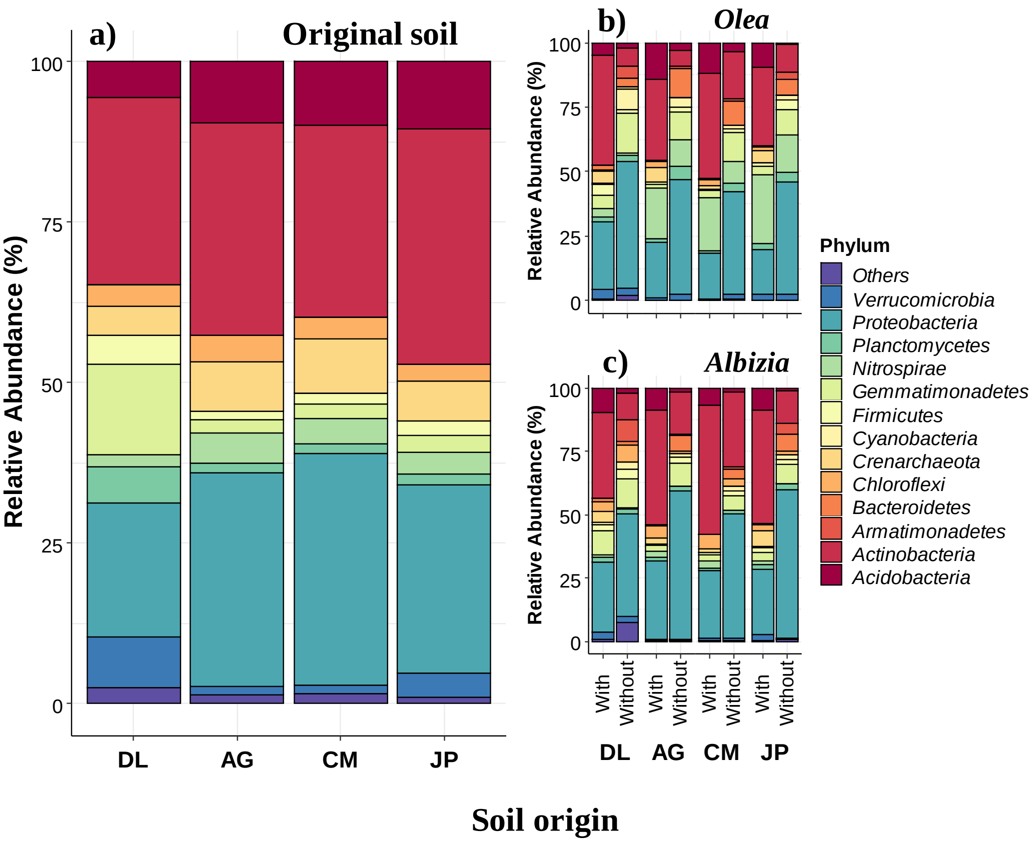

Figure 3-3. Relative abundances of (a) bacteria at the phylum level in the soil samples; and (b) before GH experiment (a) (original soil), and after GH experiment for *Olea* (b) and *Albizia* (c) seedlings with treatment (non-sterilized soil) and without treatment (sterilized soil). The values shown are means (n = 3 for soil before GH experiment and n = 8 for soil after GH experiment). DL, AG, CM and JP represent degraded land, *Albizia gummifera, Croton macrostachyus,* and *Juniperus procera,* respectively.

The values of indices (H′ and D), and the number of OTUs observed for bacteria under *Olea* (Figure 5a, e, i), and *Albizia* (Figure 5c, g, k) seedlings were significantly higher in non-sterilized than in sterilized soils. The bacterial community significantly varied among soil origin (PerMANOVA; F = 13.63, p < 0.001) and between microbial treatments (F = 3.27, p < 0.001) and seedling types (F =16.79, p < 0.001). The relative abundance of *Acidobacteria, Actinobacteria,* and *Nitrospirae* was significantly correlated with non-sterilized forest soil bacterial community (r2 = 0.63, r2 = 0.77, r2 = 0.79, respectively; p < 0.001). Proteobacteria were strongly correlated in sterilized soil (r2 = 0.63, p < 0.001), in particular for the forest soils; for the DL soil, the bacterial communities were less related to *Acidobacteria, Nitrospirae,* and *Proteobacteria*. In addition, the bacterial communities of DL were grouped in a separate cluster distant from the forest soils (Figure 6a). Among different soil properties, soil pH was strongly correlated (r2 = 0.6, p < 0.001) with the bacterial community (Figure 6a), whereas SOC, TN, C/N ratio, and available P did not show a strong correlation with the bacterial community.

3.3.1.2 Soil fungal community composition, abundance, and relationship with soil properties

In the soils before GH experiment, *Ascomycota* (48%, 7270%, 7072% and 68%) and *Basidiomycota* (21%, 16%,11% and 17%) were the most abundant fungi phyla, respectively in DL, AG, CM, and JP (Figure 3-4a) Similarly, for soils after plant, *Ascomycota* and *Basidiomycota* were the most abundant fungal phyla under *Olea* and *Albizia* seedlings (Figure 3-4b, d). *Ascomycota* had a higher relative abundance for *Olea* seedlings in non-sterilized (93%, 89%, 93%, 88%, and 76%) than in non-sterilized (81%, 80%, 78%, and 78%) DL, AG, CM, and JP soils, respectively. However, the highest and lowest abundances of *Basidiomycota* were 34% and 5% found for seedlings in DL (sterilized) and CM (non-sterilized) soils, respectively, (Figure 3-4b, d). The relative abundance of *Ascomycota* under *Albizia* seedlings in DL, AG, CM, and JP were 80%,88%, 80%, and 83%; and 62%, 62%, 75%, and 74% for seedlings in non-sterilized and sterilized soils, respectively (Figure 3-4c).

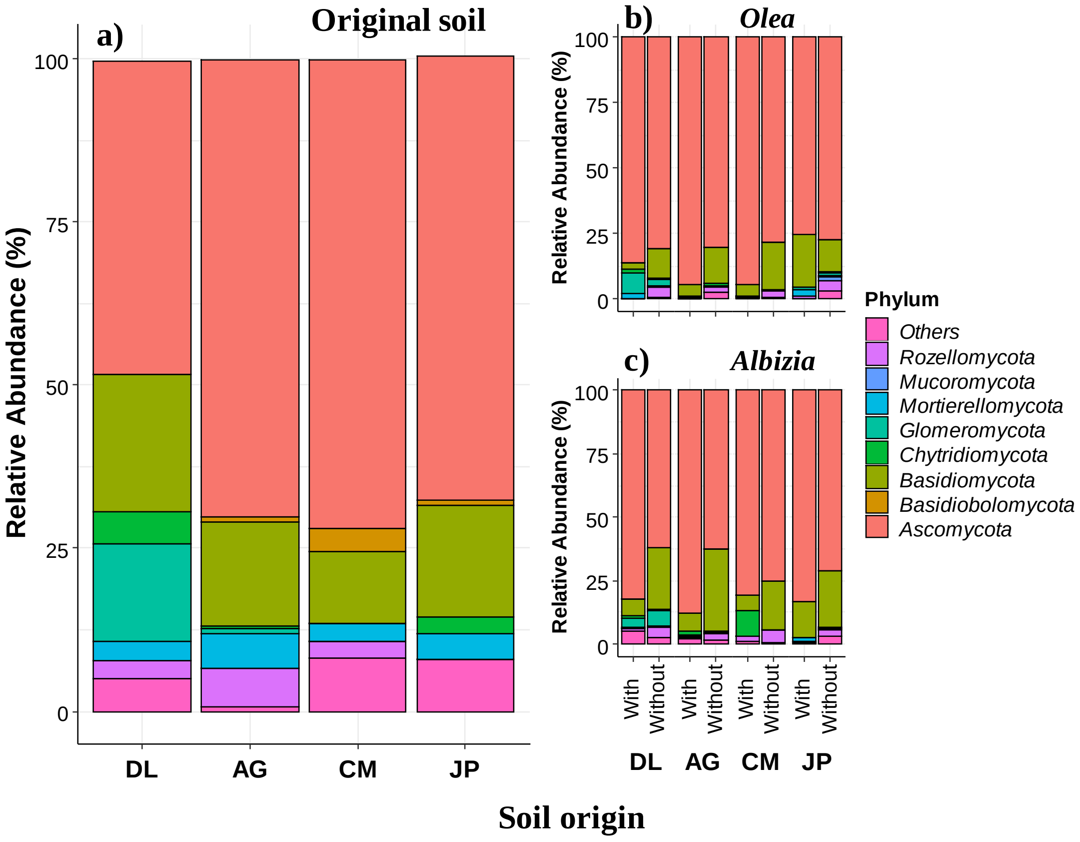

Figure 3-4. Relative abundances of fungi at the phylum level in the soil samples: before GH experiment (a) (original soil), and after GH experiment for *Olea* (b) and *Albizia* (c) seedlings with treatment (non-sterilized soil) and without treatment (sterilized soil). The values shown are means (n = 3 for soil before GH experiment and n = 8 for soil after GH experiment). DL, AG, CM and JP represent degraded land, *Albizia gummifera*, *Croton macrostachyus*, and *Juniperus procera*, respectively.

Significant differences in H′ for the fungal community were found between microbial treatments for *Olea* (Figure 3-5b) and *Albizia* seedlings (Figure 3-5d) in AG and JP soils, respectively. In addition, significant variation in D was found in CM and JP soils for *Albizia* seedlings (Figure 3-5h) between non-sterilized and sterilized soil. The number of OTUs observed for fungi were significantly higher under seedlings with non-sterilized AG soil for both *Olea* and *Albizia* (Figure 3-5j, l) than with sterilized soil, but the case was the opposite for CM soil for *Olea* (Figure 3-5j). However, the DL soil had significantly fewer OTUs for fungi than the forest soils (AG, CM, and JP).

PerMANOVA results indicated that the fungal community significantly differed among soil origins ($F = 12.71$, $p < 0.001$) and between microbial treatments ($F = 2.70$, $p < 0.001$) and seedling type ($F = 8.40$, $p < 0.001$). The relative abundance of *Ascomycota* and *Basidiomycota* had weak correlation ($r2 < 0.5$, $p < 0.001$ with the fungal community. There was no correlation between the soil properties (pH, SOC, TN, C/N ratio, and available P) and fungal community (Figure 3-6b).

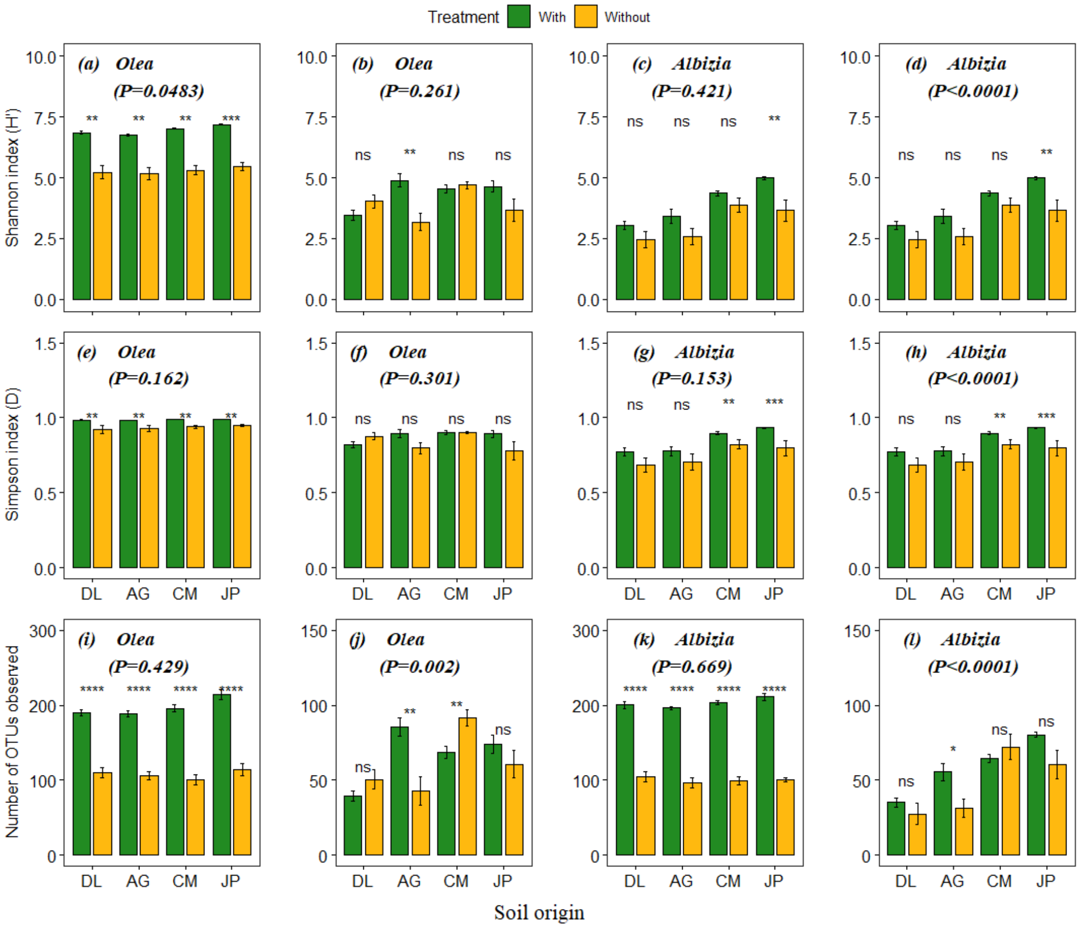

Figure 3-5. Shannon (H′) index (a–d), Simpson (D) index (e–h), and number of OTUs (i–l) observed for bacteria (columns 1 & 3) and fungi (columns 2 & 4) for Olea and *Albizia* seedlings

with (non-sterilized; green bars) and without (sterilized; yellow bars) treatments. DL, AG, CM, and JP stand for different soil origins from degraded land, or from beneath *Albizia* gummifera, *Croton macrostachyus*, *Juniperus procera*, respectively. Asterisks indicate statistically significant differences between seedlings in non-sterilized (with treatment) and sterilized (without treatment) soil (* p ≤ 0.05; ** p ≤ 0.01; *** p ≤0.001; **** p ≤ 0.0001; and ns, not significant) and P-value (Kruskal test among soil origins). Values are shown as mean ± standard error (n = 8).

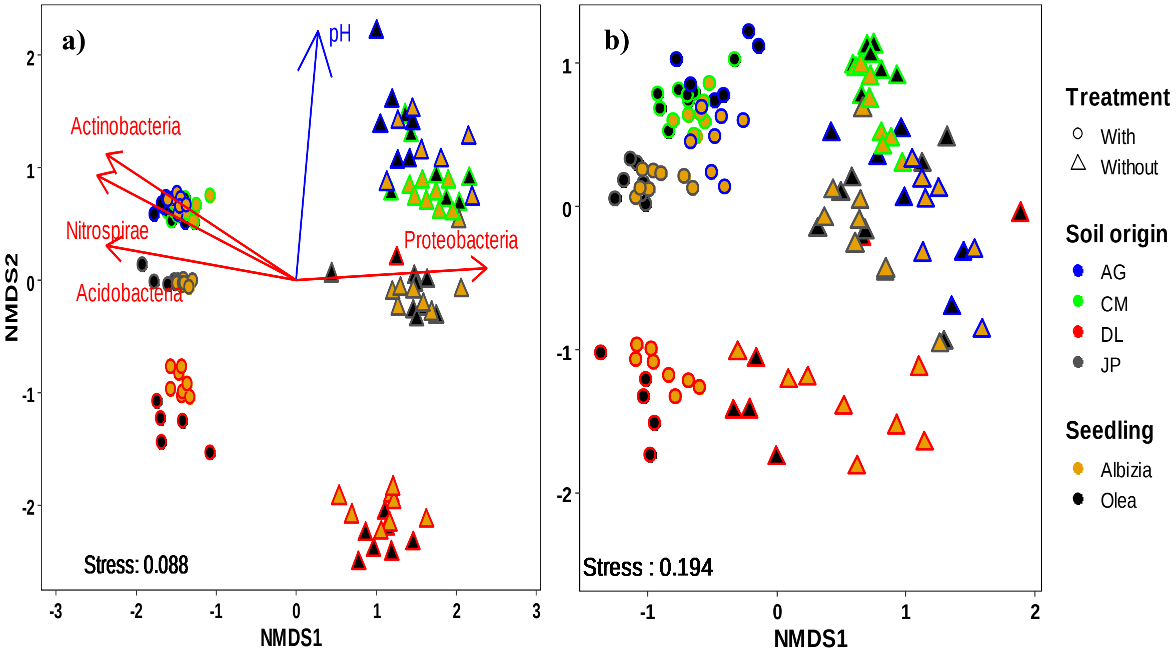

Figure 3-6. Non-metric multidimensional scaling based on Bray–Curtis dissimilarities for the (a) bacterial and (b) fungal communities. Shapes of symbols represent microbial treatments, colors of symbols represent soil origins (AG, CM, DL, and JP), and fills with the symbols represent *Albizia* and *Olea* seedlings. Arrows indicate significant correlations and the lengths of arrows are relative to the strength of the correlation. DL, AG, CM, and JP stands for soil from degraded land or from beneath *Albizia gummifera, Croton macrostachyus, Juniperus procera*, respectively.

3.3.2 Effect of soil origin and microbial treatment on plant growth and survival

The factors including species, soil origin and microbial treatments were all considered in the model for analysis of variance (Table S3). The result indicated that most of plant characteristics and soil properties are related with species, soil origin, microbial treatments, and their interactions. The results of the three-way variance analyses demonstrated that species and interactions existed between species and soil origin, microbial treatments and soil origin, and species, microbial treatments and soil origin were the most important factors affecting plant characteristics excluding the R/S ratio (Table S3). The soil origin also significantly affected plant characteristics ($P < 0.05$; Table S3). Except for root mass, microbial treatments substantially influenced plant characteristics. However, the interaction of species and microbial treatments significantly determined survival rate, shoot and total mass of plants ($P < 0.05$; Table S3).

After separated with species, the two-way variance analyses indicated that microbial treatments, soil origin, and their interaction had a significant effect on plant height, RCD, shoot mass, total mass, and survival rate of *Olea* and *Albizia* seedlings (Figure 3-8, Table S3). *Olea* seedlings grown in non-sterilized AG, CM, and JP soils showed significantly higher plant height (Figure 3-7a), total mass (Figure 3-7i), RCD (Figure S1-a), and shoot mass (Figure S1-c), than seedlings grown in sterilized soil. For the same plant characteristics, there was no significant difference between sterilized and non-sterilized DL soils (Figure 3-7a, c and e). Root mass did not vary between microbial treatments, except in CM soil, where it was significantly increased more than double in non-sterilized soil (Figure S1-e). In contrast, the R/S ratio for *Olea* seedlings grown in sterilized AG, CM, and JP soils was significantly higher than seedlings grown in the non-sterilized soils (Figure 3-7e). For *Albizia* seedlings, the RCD (Figure S1-b) and shoot mass (Figure S1-d) were significantly varied in DL and CM non-sterilized soils than seedlings in sterilized soils. The shoot mass of *Albizia* seedlings were significantly higher for both treatments of AG and non-sterilized CM soil than the other treatments (Figure S1-d). Root mass (Figure S1-f) was significantly higher in sterilized AG and JP soils, significantly lower in CM soil, and was not significantly different in DL soil

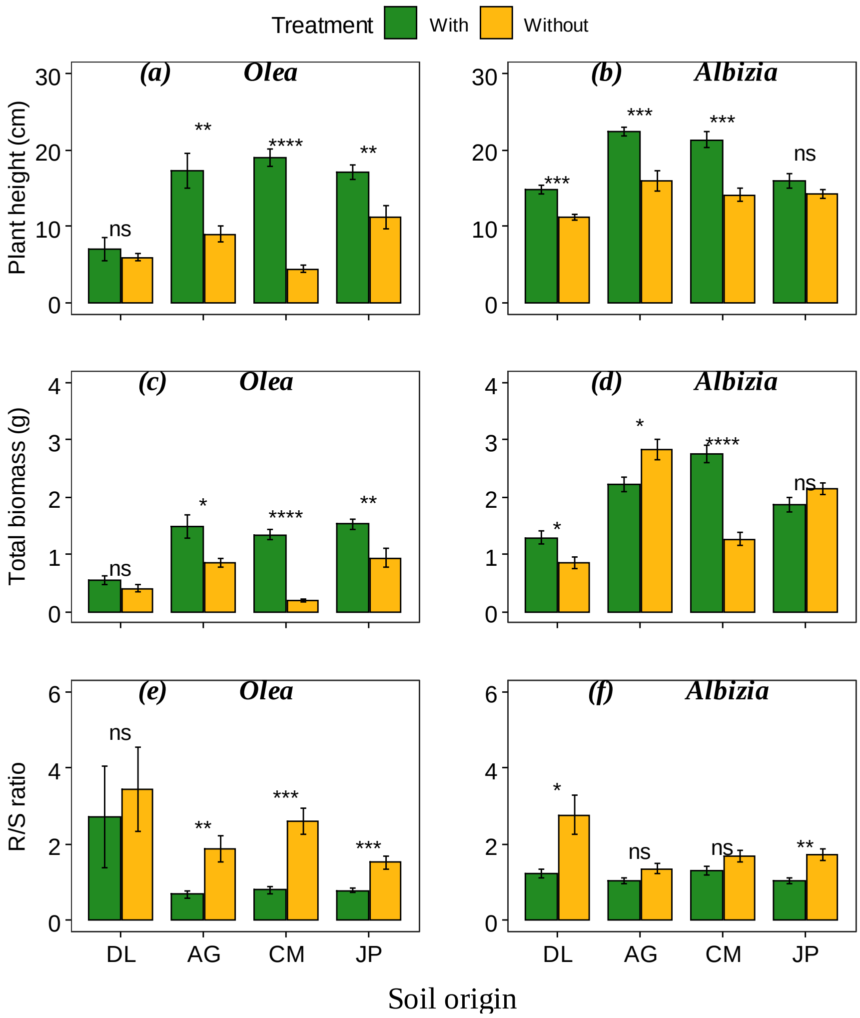

Figure 3-7. Effects of soil from different origins on plant height (a, b), total biomass (c, d), and root to shoot (R/S) ratio (e, f) of Olea and *Albizia* seedlings, respectively, with treatment (non-sterilized soil) and without treatment (sterilized soil). DL, AG, CM, and JP stand for soil origins from degraded land, or from beneath *Albizia* gummifera, *Croton macrostachyus*, *Juniperus procera*, respectively. Asterisks indicate statistically significant differences between seedlings with treatment (in non-sterilized soil) and without treatment (in sterilized soil): * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$; and ns, not significant). Values are mean ± standard error (n = 8).

Survival analysis showed no significant differences in survival rate between *Olea* seedlings with non-sterilized and sterilized soil ($\chi2(1) = 3.32$, $p = 0.061$), although the survival rate for seedlings with sterilized soil (97%) was slightly higher than for those in non-sterilized soil (95%). Survival rate significantly differed among soil origins for *Olea* seedlings ($\chi2(3) = 17.64$, $p < 0.001$). Survival of *Albizia* seedlings was significantly affected by the microbial treatments (Table S3) and soil origins (Table S3). *Albizia* seedlings survived better with the sterilized treatment (100%) than with the non-sterilized treatment (97%). Survival rate in AG, CM, and JP soils was found to be similar and significantly higher than in DL (92%). The interaction between microbial treatment and soil origin had no significant effect on survival of either *Olea* or *Albizia* seedlings (Table S3; $\chi2(3) = 3.32$, $p = 0.06$ and $\chi2(3) = 6.03$, $p = 0.42$, respectively).

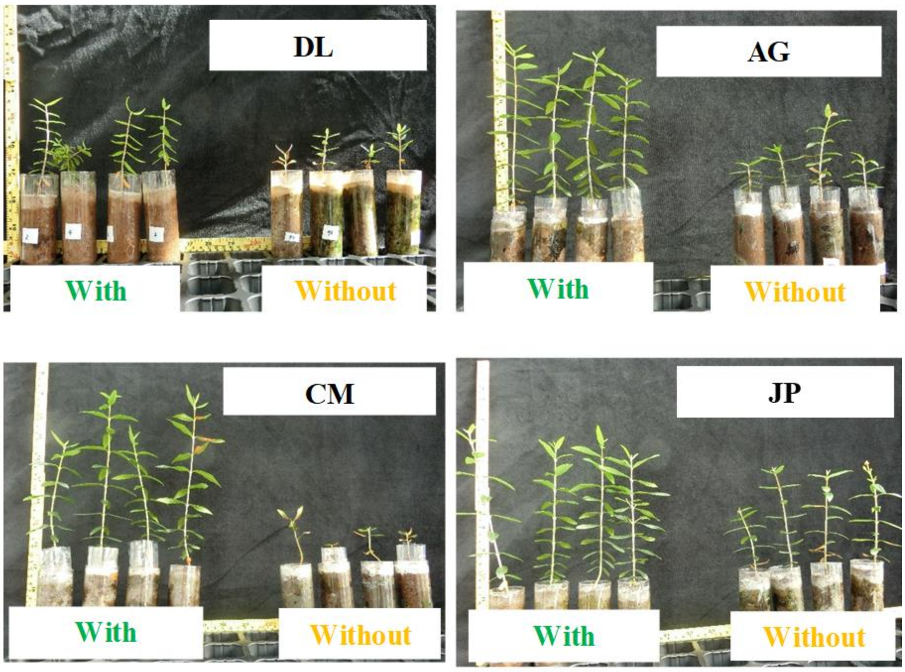

Figure 3-8. Effect of different soil origin on plant growth performance of Olea. AG: *Albizia gummifera*, CM: *Croton macrostachyus*. DL: Degraded Farmland and JP: Juniperus procera

3.3.3 Soil chemical and physical properties

Soil pH, TN content and C/N ratio were strongly dependent on species ($p < 0.05$). Soil origin ($p < 0.05$) also influenced pH, SOC, TN and available P, but not the C/N ratio. Except for available P, microbial treatments did not affect soil characteristics. In addition, the interaction between species and soil origin, and species, soil origin and microbial treatments affected SOC and TN contents (p < 0.05; Table S3). Available P and SOC content were also dependent on the interaction of species and microbial treatments, and soil origin and microbial treatments, respectively.

Significant differences ($p < 0.05$) in soil pH between original soil and the soils after the plants were observed for both species and microbial treatments in AG and JP soils (Table 3-2). SOC content for *Olea* and *Albizia* seedlings varied significantly between original soil and the soils after the plants in both microbial treatments of AG and DL soil, respectively. Whereas SOC content only varied significantly in DL (sterilized) and JP (non-sterilized) soil, respectively for *Olea* and *Albizia* seedlings. The TN contents in DL soil for both species and microbial treatments were significantly different between original soil and the soils after the plants. There was significant variation in the C/N ratio of DL (sterilized) and AG in both microbial treatments between original soil and the soils after the plants. However, Available P only significantly varied in non-sterilized AG soil of *Albizia* seedling.

Table 3-1. Major soil characteristics of degraded land and Church Forest soils (n=3).

Soil	**pH**	**MC (%)**	**SOC (%)**	**TN (%)**	**C/N**	**Avail. P (mg kg^{-1})**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**Texture class (USDA)** [a]
DL	5.6 $(0.2)^{b}$	11.7 $(1.1)^{c}$	6.0 $(0.2)^{b}$	0.8 $(0.1)^{b}$	10.2 $(0.3)^{b}$	29.4 $(1.1)^{b}$	68	11	21	Sandy clay loam
AG	6.1 $(0.1)^{ab}$	26.7 $(2.0)^{a}$	12.0 $(1.2)^{a}$	1.0 $(0.1)^{a}$	11.8 $(1.8)^{a}$	88.2 $(5.9)^{a}$	68	12	20	Sandy loam
CM	6.5 $(0.2)^{ab}$	23.3 $(1.5)^{ab}$	8.1 $(1.1)^{ab}$	0.7 $(0.1)^{b}$	11.3 $(0.4)^{a}$	39.8 $(4.2)^{b}$	64	17	19	Sandy loam
JP	7.0 $(0.3)^{a}$	17.0 $(1.6)^{bc}$	8.7 $(0.4)^{ab}$	0.8 $(0.1)^{b}$	10.8 $(0.1)^{b}$	76.3 $(5.1)^{a}$	67	14	19	Sandy loam

Different lowercase letters indicate significant differences among soil origins [values are mean (± standard error)]; Tukey HSD, $p < 0.05$, n = 3. Soil in degraded land (DL) and under mature trees of *Albizia gummifera (AG), Croton macrostachyus (CM), Juniperus procera (JP).* MC, moisture content at sampling. SOC, soil organic carbon. TN, total nitrogen. C/N, carbon/nitrogen ratio. Avail.P, available phosphorus. [a] Soil texture class is according to (Brady and Ray, 2017)

For *Albizia* seedlings, soil pH was significantly affected by microbial treatment and soil origin and their interaction, whereas for *Olea* seedlings, soil origin and its interaction with microbial treatment were significant (Table S3). Soil pH for *Olea* seedlings with non-sterilized JP soil was significantly higher than for seedlings in sterilized soil, whereas with AG soil, the opposite trend was observed (Table 2). Except for CM soil, there was a significant difference in pH between non-sterilized and sterilized soil for *Albizia* seedlings, i.e., in DL, AG, and JP soils, pH was significantly higher for seedlings in sterilized soil (Table 3-2). For both *Olea* and *Albizia* seedlings and regardless of microbial treatments, pH of DL was significantly lower than AG and CM but was comparable with JP (Table 3-2).

Generally, soil SOC, TN, C/N ratio, and available P for *Olea* seedlings were not significantly affected by microbial treatment, soil origin, and their interaction. SOC, TN, and C/N ratio for *Olea* seedlings did not significantly vary between soil origins (Table S3). Sterilized AG soil had a higher SOC (10.10%) and TN (0.92%) contents, whereas the lowest SOC (4.48%) and TN (0.40%) contents were found in non-sterilized DL soil (Table 2). The highest (11.40) and the lowest (10.90) C/N ratios for *Olea* were measured in CM sterilized and non-sterilized soils, respectively (Table 3-2).

Soil available P significantly differed among soil origins for *Olea* seedlings (Table S3). The non-sterilized AG soil had a significantly higher available P (88.00 mg kg^{-1}) than the others. The lowest content of available P (28.40 mg kg^{-1}) was found in DL non-sterilized soil for *Olea*. Available P in the forest soils was highest in AG followed by JP then CM (Table 3-2). In contrast to *Olea* seedlings, soil SOC, TN, C/N, and available P for *Albizia* seedlings significantly varied among soil origins, with SOC and TN in particular being significantly influenced by soil origin.

However, except for available P content and C/N ratio, other soil characteristics did not significantly differ between sterilized and non-sterilized soil (Tables 3-1 and 3-3). SAS was numerically higher in non-sterilized than in sterilized soil for both *Olea* and *Albizia* seedlings (Table 2), significantly so for AG and CM soil with *Olea* seedlings, and for AG soil with *Albizia*. Regardless of microbial treatment, for both *Olea* and *Albizia* seedlings, SAS for all of the forest soils (AG, CM, and JP) was significantly higher than for DL soil (Table 3-2).

Table 3-2. Soil characteristics of *Olea* and *Albizia* seedlings inoculated with and without four different soil origins at the end of the 5 months experiment (DL, soil from degraded land; AG/CM/JP, soil from beneath *A. gummifera, C. macrostachyus*, and *J. procera*, respectively).

Species	**Soil origin**	**pH**		**SOC (%)**		**TN (%)**		**C/N**		**Avail.P (mg kg^{-1})**		**SAS (SI, cm^{-1})**	
		with	**without**	**with**	**without**	**with**	**without**	**with**	**without**	**with**	**without**	**with**	**without**
Olea	DL	5.9	5.9	4.5	7.9	0.4	0.70	11.0	11.0	28.4	30.7	0.02	0.01
		(0.1)^{cA}	(0.1)^{cA}	(1.3)	(0.8)	(0.1)	(0.1)	(0.2)	(0.1)	(0.6)^{d}	(0.4)^{d}	(0.0)^{c}	(0.0)^{c}
		ns	ns	ns	*	*	*	ns	ns	ns	ns		
	AG	6.7	7.2	6.7	10.1	0.6	0.92	11.1	11.4	88.0	86.1	0.05	0.04
		(0.1)^{aB}	(0.1)^{aA}	(1.8)	(1.5)	(0.2)	(0.1)	(0.5)	(0.2)	(3.3)^{a}	(4.7)^{a}	(0.0)^{aA}	(0.0)^{aB}
		*	*	*	*	*	ns	ns	ns	ns	ns		
	CM	6.6	6.6	6.3	5.7	0.6	0.50	10.9	11.4	42.2	42.5	0.04	0.03
		(0.1)^{aA}	(0.1)^{aA}	(1.4)	(1.5)	(0.1)	(0.1)	(0.2)	(0.2)	(0.7)^{c}	(0.7)^{c}	(0.0)^{bA}	(0.0)^{bB}
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
	JP	6.3	5.9	8.3	6.8	0.7	0.62	11.3	10.9	77.9	74.8	0.04	0.03
		(0.1)^{bA}	(0.1)^{bB}	(1.7)	(0.7)	(0.1)	(0.1)	(0.1)	(0.1)	(2.2)^{b}	(2.0)^{b}	(0.0)^{b}	(0.0)^{b}
		*	*	ns	ns	ns	ns	ns	ns	ns	ns		

Albizia	DL	5.6 (0.1)^{dB} ns	5.9 (0.0)^{dA} ns	3.9 (0.0)^{d} *	3.7 (0.0)^{d} *	0.4 (0.0)^{d} *	0.3 (0.0)^{c} *	10.1 (0.1)^{a} ns	11.4 (0.2)^{a} *	27.3 (0.4)^{dB} ns	28.6 (0.6)^{dA} ns	0.02 (0.0)^{d}	0.01 (0.0)^{c}
	AG	7.0 (0.1)^{aB} *	7.4 (0.0)^{aA} *	12.1 (0.2)^{a} ns	11.9 (0.7)^{a} ns	1.0 (0.0)^{a} ns	1.0 (0.1)^{a} ns	11.6 (0.1)^{a} *	11.6 (0.1)^{a} *	74.1 (2.0)^{bB} *	86.9 (3.9)^{bA} ns	0.05 (0.0)^{aA}	0.04 (0.0)^{aB}
	CM	6.5 (0.0)^{bA} ns	6.6 (0.1)^{bA} ns	8.3 (0.1)^{b} ns	8.6 (0.4)^{b} ns	0.8 (0.0)^{b} ns	0.8 (0.0)^{b} ns	10.7 (0.1)^{b} ns	10.8 (0.1)^{b} ns	34.5 (0.8) ^{c} ns	47.3 (1.1)^{c} ns	0.04 (0.0)^{b}	0.04 (0.0)^{a}
	JP	6.0 (0.0)^{cB} *	6.3 (0.0)^{cA} *	7.3 (0.1)^{c} *	7.7 (0.1) ^{c} ns	0.7 (0.0)^{c} ns	0.7 (0.0) ^{b} ns	10.6 (0.1)^{b} ns	10.7 (0.0)^{b} ns	74.2 (3.7)^{aB} ns	94.8 (1.5)^{aA} *	0.03 (0.0)^{c}	0.03 (0.0)^{b}

Different superscript lowercase letters indicate significant differences among soil origin for each species; different superscript uppercase letters indicate significant differences between treatments (Tukey HSD, $p < 0.05$, n = 8) and asterisks indicate statistically significant differences between original soil (Table 1) and soil after plants in the same soil type (* $p \leq 0.05$; and ns, not significant). Values are mean (± standard error).

3.4 Discussion

The soil from the four origins in this study caused significant variation in plant characteristics (Figures 3-7, 3-8). *Olea* seedlings with non-sterilized soil of *Albizia gummifera*, *Croton macrostachyus*, and *Juniperus procera* showed higher plant growth compared to seedlings with sterilized soil. Plant characteristics of *Albizia* seedlings were consistently affected by non-sterilized soil from *Croton macrostachyus*, whereas for other soil origins the effect was not consistent. *Olea* and *Albizia* are species that co-exist in dry Afromontane forest of Ethiopia (Aynekulu et al., 2011) and have arbuscular mycorrhizal associations (Wubet et al., 2003). Our results agree with other studies that have shown that inoculation with the appropriate microbes can significantly modify or improve seedling growth (Wubet et al., 2003), pathogen resistance (Wehner et al., 2010), and biomass production (Zhang et al., 2011). Conversely, seedlings of both species in non-sterilized *Croton macrostachyus* soil consistently varied in plant growth (Figure 3-7). This may be linked to the abundance of *Actinobacteria* and *Nitrospirae* for seedlings in non-sterilized soil, which is strongly related to the bacterial community of forest soil in particular with *Croton macrostachyus* soil (Figure 3-6). Several reports have indicated that members of the phylum *Actinobacteria* are involved in organic matter decomposition, plant growth promotion, and soil pathogen control (Zhang et al., 2011). Also, *Nitrospirae* is one of the phyla whose members are involved in soil nitrification (Altmann et al., 2003); therefore, their abundance in soil may influence the availability of soil nitrogen (Li et al., 2018). However, the phylum *Proteobacteria* was highly correlated with seedlings in sterilized soil. Furthermore, relatively high soil pH was observed for seedlings in non-sterilized soils (Table 3-2 and Figures 3-6). Studies have reported that the abundance of *Proteobacteria* is positively correlated to soil pH (Li et al., 2018). In addition, lower forest soil TN (0.07%) and P (39.84 mg kg^{-1}) contents were found in *Croton macrostachyus* soil. According to Wubet et al. (2003) , native tree species of the dry Afromontane forest in Ethiopia have mycorrhizal associations, which are effective tools when the soil nutrients (i.e., N and P) are limited (Wubet et al., 2003). However, arbuscular mycorrhizal abundance in the experimental soil was low, and fungal communities did not correlate with the soil properties, and had lower diversity, evenness, and number of operational taxonomic units than bacterial communities. This result is in line with other studies (Smith et al., 2011; St-Denis et al., 2017). Thus, when a difference in pH range preference for optimum growth pertains, soil pH is often a factor exerting more control over the abundance of the bacterial community than the fungal community. Additionally, the beneficial effect of soil from beneath *Croton macrostachyus* on the regeneration of *Olea* seedlings (Abiyu et al., 2017) has been reported in the highlands of Ethiopia.

As expected, *Olea* seedlings in degraded land soil did not vary in size among microbial treatments (Figure 3-7). This could be due to the low level of soil microbial diversity and abundance, which are common soil characteristics of degraded land (Asmelash et al., 2016). Soil microbes widely known to enhance plant growth, increase efficiency of nutrient uptake, facilitate establishment and competitive ability of seedlings. Moreover, in the present study, degraded land soil had lower fungal diversity and number of operational taxonomic units than forest soil. Correspondingly, SOC, TN, available P, and moisture content were found at lower levels in degraded land soil than in forest soil (Table 3-1).

Plant biomass allocation strategy is species-dependent and varies with environmental factors (Asmelash et al., 2016). Studies have shown that resource availability controls biomass allocation patterns in plants (Atkin et al., 2006; Jiang and Wang, 2017), especially for root to shoot ratio. In the present study, root to shoot ratios for *Olea* seedlings in *Albizia gummifera, Croton macrostachyus* and *Juniperus procera* sterilized soil were higher than in non-sterilized soil. The root to shoot ratio was highest in *Olea* seedlings, reaching 3.44 in degraded land soil, a value not influenced by soil sterilization. This could be because plants under conditions of low soil nutrients and limited water are obliged to allocate high biomass to their roots to exploit the soil resources more effectively (Classen et al., 2015; Eziz et al., 2017). In contrast, low root to shoot ratios were found in seedlings in non-sterilized forest soils, which could be because seedlings in non-sterilized soils have greater access to water and nutrients, provided by the microbial association, meaning that seedlings were able to allocate more biomass to the shoot. A similar finding was also reported by Zandavalli et al. (2004).

Soil aggregate stability and the process of structure formation are complex, influenced by soil properties (e.g., clay content, organic matter), plant root development, and soil microbial activity (Rashid et al., 2016). Soil aggregate stability is an indicator of soil aeration and nutrient availability, soil erosion resistance, root penetration, and water regime of the soil (Rashid et al., 2016). In this study, soil aggregate stability was significantly higher for forest soils than degraded land soil. This result is in agreement with the findings by Delelegn et al. (2017), who reported higher soil aggregate stability in natural forest soil than degraded croplands in the highlands of northern Ethiopia. A similar result was also reported by Caravaca et al. (2004). SOC was also higher in forest soil than degraded land in this study. SOC is the main element in soil aggregate formation and is directly related to soil microbial diversity Caravaca et al. (2004). Loss of SOC results in significant deterioration in soil structure (Daynes et al., 2013), which is a key indicator of soil degradation (Daynes et al., 2013). Moreover, loss of beneficial soil microbes (mainly *fungi* and *bacteria*) significantly affects soil aggregate stability (Obalum et al., 2017). Furthermore, fungi play a significant role in endorsing the formation of macro-aggregates through their hyphae, which “glue” the micro-aggregates together (Blankinship et al., 2016). However, as mentioned above, the degraded land soil had lower fungal diversity than forest soil. Thus, greater soil aggregate stability under seedlings with non-sterilized *Albizia gummifera* and *Croton macrostachyus* soils (Table 3-2) can be attributed to the higher abundance of beneficial microbes (Figure 3-3) that facilitate the formation of micro and macro-aggregates (Bearden, 2001).

3.5 Conclusions

Higher growth in non-sterilized, than sterilized forest (*Albizia gummifera, Croton macrostachyus and Juniperus procera*) soil indicates a microbial benefit to seedling growth from forest soil. I also observed higher plant growth in forest soils than in degraded soils mainly due to higher relative abundance of beneficial bacterial phyla (*Acidobacteria*, *Actinobacteria*, and *Nitrospirae)*. Soil pH showed a strong correlation with the abundance of the bacterial community, but no relationship was found between soil properties and fungal communities. Moreover, the effect of soil microbiome was noticeable on the performance of *Olea* seedlings grown in the soil from *Croton macrostachyus*. This suggests that soils from *Croton macrostachyus* can promote growth and survival of *Olea* and *Albizia* seedlings in degraded lands. Overall the results of this study implies that soils from the remnant Church Forests could serve as a potential source of soil microbiome for the restoration of degraded lands using native tree species.

Chapter 4

Role of Church Forest soil in assisting seedling establishment under a degraded land condition

4.1 Introduction

Soil microbial communities affect plants growth and performances through direct and indirect interactions (Lance et al., 2019). Soil microbes play key roles in the transformation of nutrients into inorganic forms available for plant assimilation. Moreover, introduction of bacterial and fungal inocula can exploit, translocate, mineralize and mobilize soil P, K, Fe reserves, increase organic matter or fix N from the atmosphere (Rashid et al., 2016). Several studies also revealed that these beneficial microbes can enhance drought tolerance, and pathogen resistance of the host plant (Wehner et al., 2010; Essahibi et al., 2018). However, these microbial communities are mostly affected by degradation including soil disturbance (soil erosion), change in chemical and physical properties of soil (Araujo et al., 2014; Blankinship et al., 2016). Particularly, the loss of the top soil layer significantly alters the soil microbial community and interrupt the ecosystem functions. Therefore, the inoculation of soil microbial communities has become a widely used measure intended to improve plant establishment in ecological restorations (Wubs et al., 2016; Neuenkamp et al., 2019). A common technique of manipulating soil microbes is to apply soil collected from a reference plant community (St-Denis et al., 2017; Lance et al., 2019). In many degraded landscapes due to sever degradation, getting a reference plant community for soil microbial inoculation purpose has been a main challenge (Singh, 2015). Thus, in such cases commercially available soil microbes often used alternatively for potted plants and/or in the soil of the restoration sites (Ambrosini et al., 2015; Lance et al., 2019). Whereas in case of Ethiopia, small patches of forest are existing in the compounds of Ethiopian orthodox Tewahido churches and monasteries (Wassie et al., 2009b; Abiyu et al., 2011). These forests called “Church Forests”, which constitute the last remnants of the original forest cover in Ethiopia, having been conserved for more than a century. Church Forests are foci of vegetation and soil microbial biodiversity (Wassie et al., 2009a). However, the role of microbial communities from Church Forests on the early stages of native trees establishment in degraded lands still unknown. Therefore, this study was designed to address the role soil microbial from Church Forest in assisting seedling of *Olea europaea* establishment under degraded land conditions.

4.2 Material and methods

4.2.1 Site description

Please see 3.2.1

4.2.2 Soil collection

At the same day of the seedling plantation, soil samples were collected under the canopy of three predominant native tree species: *Albizia gummifera* (AG), *Croton macrostachyus* (CM), and *Juniperus procera* (JP), in the Church Forest (Figure 3-1c), and from adjacent degraded farmland (DL). Soil samples from each tree were mixed to obtain a composite inoculum.

4.2.3 Study species

Olea europaea L. subsp. cuspidata is one of the most important native tree species of Ethiopia. The specie was selected for this study based on their social and ecological importance and its limited survival and regeneration ability in degraded lands of the Ethiopian highlands (Wassie et al., 2009b; Wassie et al., 2009a). *Olea europaea subsp. cuspidata* (Wall. ex DC.) is a late-successional evergreen tree species found in dry Afromontane forest between 1250 and 3100 m a.s.l. (Friis, 1992). The species is hardy and drought resistant once established, even in poor soils; adult trees are commonly 15-25 m high (Tesemma, 2007).

4.2.4 Experimental design

In the degraded land, three blocks (12 m x 6 m) were randomly selected and fenced at three different places for seedling growth and survival experiment (Figure 4-1). Each block was divided into two plots (5 m x 5m) one of which was assigned for assisted seedling establishment

(AR) and the other for non-assisted seedling establishment (NR). Before the experiment, *Olea* seedlings were raised in a nearby nursery. The nursery site has similar climate conditions as the experiment site. A total of 96 seedlings (<1–year old) were used for this experiment. In July 2018, 16 seedlings per plot were planted in 1m x 1m spacing. For each seedling in assisted plots, around 1 kg of Church Forest soil was applied during the planting time.

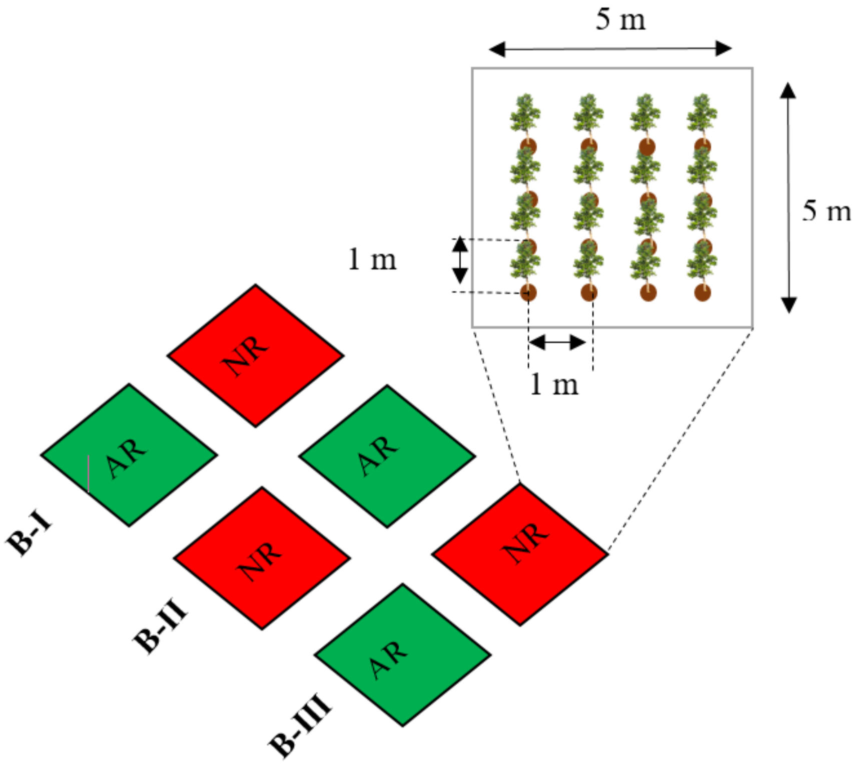

Figure 4-1. The layout of the experiment and trees seedlings arrangement in a plot.

4.2.5 Data collection

Seedling survival counts, plant height and root collar diameter were collected every month for 21 months. Plant height and root collar diameter were measured using meter tape and caliper, respectively.

4.2.6 Data analysis

Assumptions of the normal distribution of residuals, homogeneity of variances were tested, non-normal data were transformed with square root transformation to meet the assumption. Two–way ANOVA was used to test significance effects of treatments and months on plant height, root collar diameter and survival rate. The difference between assisted (AR) and non-assisted (NR) treatments in plant height, root collar diameter and survival rate at the end (21-months) was analyzed with paired t-test. All statistical analyses were performed in R (Team, 2016) using the interference implemented RStudio (version 1.1.383).

4.3 Results

4.3.1 Seedling growth

The analysis of variance result showed that soil treatments, growth period (month) and significantly affected plant height (Table 4-1, Figure 4-2), however, their interaction was not significantly affected the plant height (Table 4-1). Assisting with church soil significantly affected the height of seedlings: assisted seedlings grew better than non-assisted ones (Figure 4-2a). The height of assisted seedlings varied from 77–93 cm at 21 months (Figure 4-2c).

Table 4-1. Effects of treatment and month on plant height, root collar diameter and survival rate.

Factors	*df*	*p*-value		
		Plant height	Root collar diameter	Survival rate
Treatment	1	0.008	0.272	0.005
Month	6	0.006	<0.001	<0.001
Treatment × month	6	0.895	0.425	0.530

Note: treatments are Assisted and non-assisted *Olea* seedlings, months refers to the growing periods.

Growth period (month) significantly affected the growth of root collar diameter (Table 4-1). However, there was a significant difference in root collar diameter between soil treatments and their interaction with the growth period (month). The seedlings root collar diameter result showed no significant difference between assisted and non-assisted ones at 21 months. The root collar diameter also showed a retarded growth for both treatments from 3–12 months and increased over 15 months.

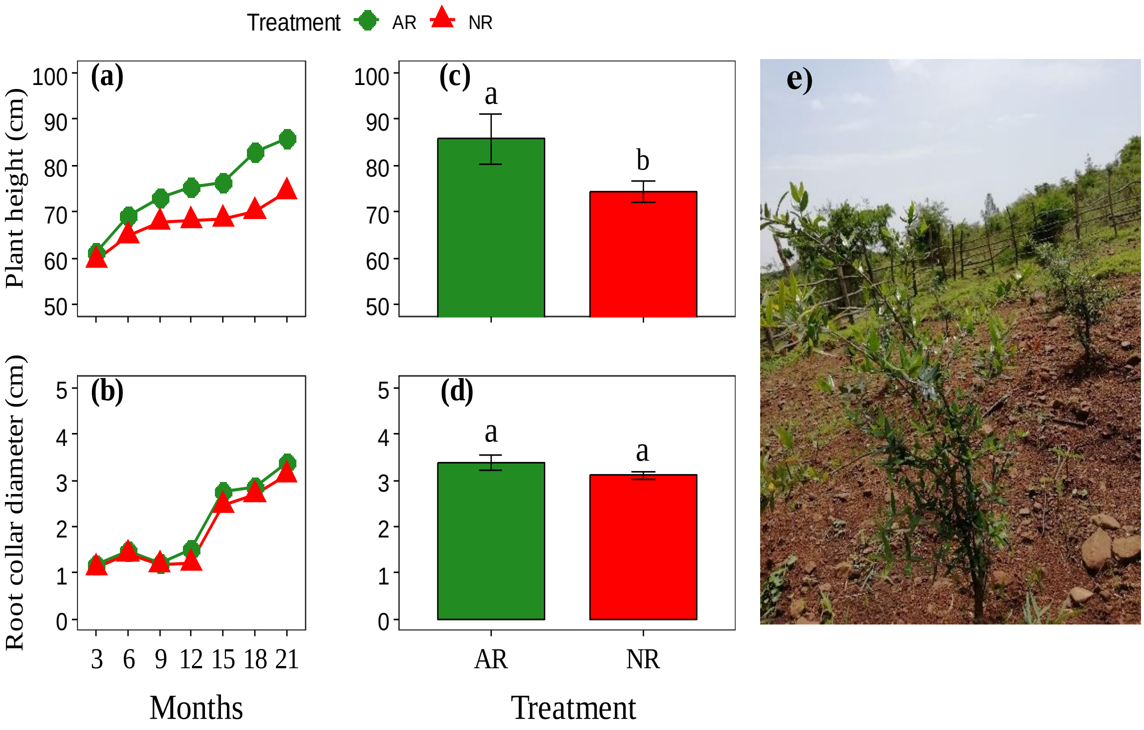

Figure 4-2. Growth of Olea seedlings in height and root collar diameter across months (a, b) and at 21-months (c, d); assisted (AR) with Church Forest soil and non-assisted (NR). Bars are mean value ± standard deviation, different letters indicate a significant difference between treatment (P< 0.05). Image of assisted *Olea* seedlings (e) was taken after 13 months of planting.

4.3.2 Seedling survival

Soil treatment and growth period (month) significantly affected the survival of seedlings (Table 4-1). The result also showed soil treatment and growth period (month) did not interact. Survival was significantly higher for assisted seedlings than non-assisted ones (4-3a). The high mortality of non-assisted seedlings was observed from month 3-6 and 9-12 of the growth period. The survival was 89.7% and 77.3% for assisted and non-assisted seedlings at 21 months, respectively.

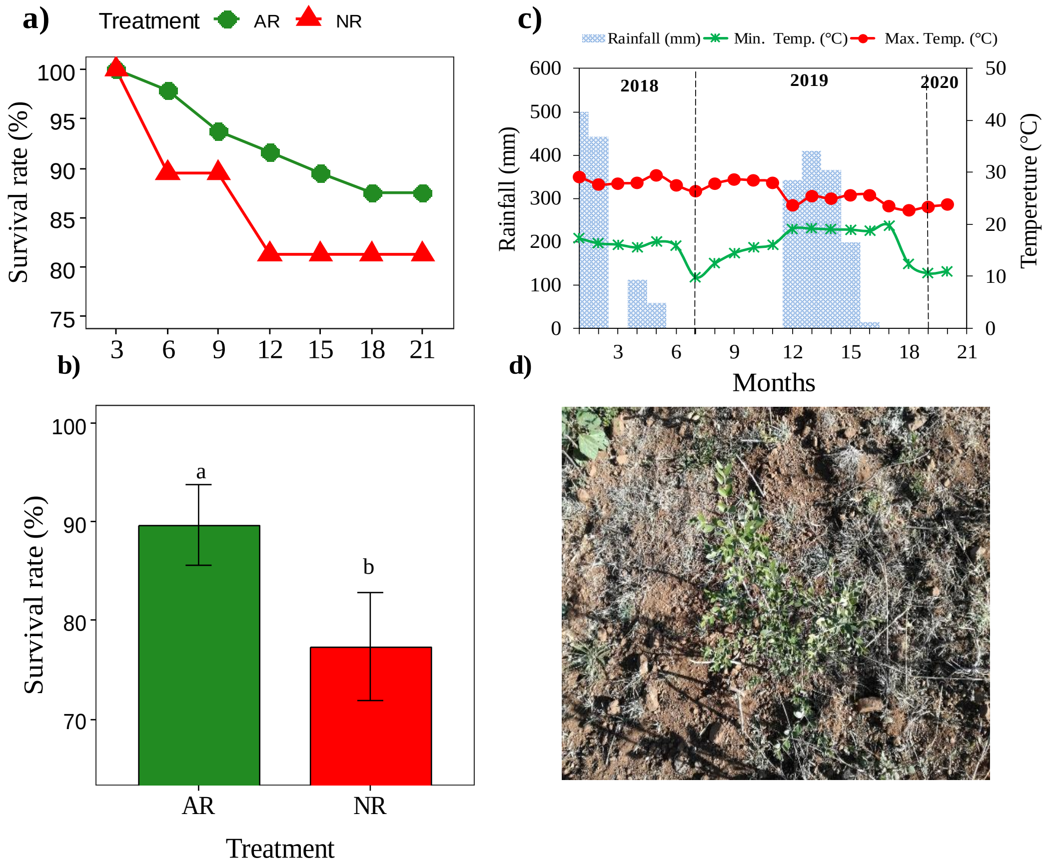

Figure 4-3. Survival of Olea seedlings (a) across months and at 21 months of experiment (b); assisted (AR) with Church Forest soil and non-assisted (NR). Bars are mean value ± standard deviation, letters indicate different letters significance difference (P<0.05). Climate condition (mean rainfall, maximum and minimum temperature) during the growth period (c). Image of assisted *Olea* seedling (d) was taken after 7 months of planting.

4.4 Discussion

The topsoil layer is an important source of seed, nutrients, and soil microbes in terrestrial ecosystems (Ferreira and Vieira, 2017; Maiti and Ahirwal, 2019). This soil in degraded lands is usually lost and damaged (Kooch et al., 2018). Particularly, the loss of soil microbes significantly affects the recovery of vegetation in degraded land. Several studies have reported that the soil community is an important driver of plant community development in degraded lands (Wubs et al., 2016). Tree survival and field establishment is a significant factor in the restoration of degraded land (Asmelash et al., 2016). In this study, *Olea* seedling inoculated with intact soil form Church Forest clearly showed high growth and survival than seedling without Church Forest soil in degraded land condition. Soil microbes increased plant growth and productivity (St-Denis et al., 2017), by enhancing resource availability for the host plant including protection from biotic factors (Essahibi et al., 2018). Moreover, soil microbes such as fungi (i.e., AM) can mobilize nutrients in the soil and transfer these nutrients to the host plants (Siddiqui and Futai, 2008). Studies also confirmed that seedlings inoculated with microbes had higher growth than non-inoculated seedlings (Sousa et al., 2012; Urgiles et al., 2014). In addition, seedling stages of plants are usually more sensitive to environmental or edaphic constraints than the adult stages (Muñoz-Rojas et al., 2016). Limited availability of nutrients (i.e., N and P) and soil moisture major challenge for seedlings establishment in degraded lands (Lance et al., 2019; Tulod and Norton, 2019). In this study, we found that the survival rate of *Olea* seedlings was lower under non-assisted ones. Seasonal variations in water availability in soil, which can increase seedling mortality in sites during the dry period. Soil microbes, in particular, fungi have a high ability to adapt environmental changes during drought conditions and long term stresses (Kannenberg and Phillips, 2017). Therefore, the association with this microbe allowing the plants to have a wider range of possibilities to adapt and survive (Vimal et al., 2017).

4.5 Conclusion

In conclusion, we have confirmed that the soil from the remnant Church Forest affects the development of *Olea* seedlings growth and survival under degraded land condition. Moreover, we found that soil inoculum from Church Forest had a higher positive impact on the plant height of *Olea* seedlings and also high survival rate during dry seasons. However, non-assisted seedlings had lower growth and high mortality than assisted ones. Therefore, Church Forest soil can enhance the seedling establishment of native tree species in degraded land.

Chapter 5

General conclusions and recommendations

5.1 General conclusion

This study demonstrated that both SOC and TN stocks varied significantly across the land uses, topographic positions, and agro-ecosystems. SOC and TN stocks were significantly higher in bushland (166.22 Mg ha^{-1}) and grazing lands (13.11 Mg ha^{-1}) at Guder. The lowest SOC and TN stocks were observed in cropland (25.97 and 2.14 Mg ha^{-1}) at Aba Gerima, which was mainly attributed to frequent and unmanaged plowing and extensive biomass removal. Compared to other land uses, plantations exhibited lower SOC and TN stocks due to poor undergrowth and overexploitation for charcoal and firewood production. Each of the three sites showed distinct characteristics in both stocks, as indicated by variations in the C/N ratios (11–13 at Guder, 10–21 at Aba Gerima, and 15–18 at Dibatie). Overall, land use was shown to be an important factor influencing the SOC and TN stocks, both within and across agro-ecosystems, whereas the effect of topographic position was more pronounced across agro-ecosystems than within them. Specifically, Aba Gerima had lower SOC and TN stocks due to prolonged cultivation and unsustainable human activities, thus revealing the need for immediate land management interventions.

This study was aimed to evaluate the soil microbial potential of soils from Church Forest in the northwest highlands of Ethiopia and its effect on seedling growth of two selected native tree species (*Olea europaea* and *Albizia gummifera*). The results showed significantly higher plant height, root collar diameter, shoot and total mass for seedlings grown in non-sterilized forest soils than those grown in sterilized soil. Furthermore, the relative abundance of Acidobacteria, Actinobacteria, and Nitrospirae was significantly correlated with non-sterilized forest soil bacterial community (r2 = 0.6–0.8, $p < 0.001$). Soil pH had a strong effect on the abundance of the bacterial community in the Church Forest soils. Overall, seedlings grown in Church Forest soils showed better performance and survival rate, because of higher soil microbial abundance and diversity, than those grown in soil from degraded land. This study further demonstrated that the effect of soil microbiome was noticeable on the performance of *Olea* seedlings grown in the soil from *Croton macrostachyus*. This suggests that soil from remnant Church Forests, particularly soil from beneath *Croton macrostachyus*, serves as a good soil origin and promotes native tree seedling growth and survival in degraded lands.

This study aimed to evaluate the role soil microbial from Church Forest in assisting seedling of *Olea europaea* under degraded land conditions in Aba Gerima. Plots assisted with intact Church Forest soil and without were arranged in randomized block design with three replicates. In each plot, 16–*Olea* seedlings were planted in 1m x 1m spacing. The result showed that *Olea* seedlings assisted with intact soil form Church Forest had high growth and survival than seedlings without Church Forest soil in degraded land condition. The height of assisted seedlings was varied from 77–93 cm at 21 months. The high mortality of non-assisted seedlings was observed from month 3–6 of the growth period. The survival was 89.7% and 77.3% for assisted and non-assisted seedlings, respectively. The result of this study confirms that soil microbes from Church Forest can assist to facilitate the seedling establishment of native tree species in degraded lands.

5.2 Recommendations for further research and application

- This study also found that among the three watersheds, Aba Gerima was found under a severely degraded situation owing to prolonged cultivation and unsustainable human activities, thus revealing the need for immediate land management interventions.

- This study confirms that Church Forest soils are endowed with beneficial native microbes (mainly bacteria and fungi), which can serve as a source for native soil inoculum for native restoration in degraded land. This result needs further studies to identify the most important to soil microbes across Church Forests.

- This study confirms that soil from Church Forest can facilitate the growth and survival of native trees seedlings establishment in degraded land.

- Continuous, use of a bulk soil from the remnant Church Forest may lead to irreversible loss of this important forest resource. Therefore, further artificial production is necessary to develop a sustainable supply of soil inoculum for native tree restoration in the region.

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Summary

Land degradation is the top global concern identified in the 2015 Status of the World's Soil Resources report. In Ethiopia, due to its prolonged and unsustainable agricultural history, land degradation has been causing the most severe problem on agricultural production and other ecosystem services. On the other hand, there have been efforts made on countermeasures that include afforestation, and establishment of exclosures (protecting human and animal interference) implemented in different parts of the country. Though such efforts (i.e., exclosures) have improved soil fertilities and vegetation covers, achieving restored ecosystem function and diversity through the establishment of native tree species has proved a challenging task due to the slow nature of the process and poor availability of beneficial soil microbes in the degraded lands. Whereas small patches of natural forest called "Church Forests", which constitute the last remnants of the original forest cover in Ethiopia, having been conserved for more than a century. These Church Forests, on top of their social and spiritual values, they are foci of biodiversity being used for seed sources and germplasm conservation sites for native fauna and flora.

However, scientific information on key ecological indicators of land degradation or afforestation such as soil organic carbon (SOC), total nitrogen (TN) stocks are scarce to prioritize and design evidence-based land management interventions. Moreover, there is lack of studies characterizing the role of microbial communities or evaluating the inoculum potential and the effects of microbes from Church Forests on the early stages of native trees establishment in degraded lands.

Thus, the overall objective of this research is to contribute for the assisted-restoration of native trees in degraded lands through using Church Forest soils as a source of native microbes. The specific objectives were to: 1) prioritize severely degraded areas for management intervention

through assessing SOC and TN stocks in different land uses and topographic positions found in three agroecosystems of the Upper Blue Nile basin; 2) to clarify the soil microbial potential of Church Forest soil and its effect on seedling growth of selected native trees species under glasshouse conditions and 3) to evaluate the role of Church Forest soil in assisting the seedling establishment of a selected native tree under a degraded land field conditions. This thesis consists of five chapters as described below:

Chapter 1 explains the introduction section of the study. It presents the study background, problem statement, objectives and description of the study area. Moreover, it provides an overview of land degradation and deforestation, ecological restoration efforts, the seedling establishment in degraded lands based on literature review. It then describes the aim of this study and the overall structure of the thesis.

Chapter 2 presents the SOC and TN stocks in different land uses, topographic positions of the three selected watersheds found across three agro-ecosystems of the Upper Blue Nile basin. The studied watersheds; namely, Guder, Aba Gerima and Dibatie represent the highland, midland and lowland agroecosystems of the basin, respectively. A total of 352 soil samples taken from 4–land uses, 3–topographic positions and 3–soil depths in the three watersheds were analyzed for SOC and TN. I found that land use, topographic positions, agroecosystems and their interactions have a significant influence in both stocks. Not surprisingly, the cropland located in the upper topographic position was found the most degraded land use type, likely due to higher erosion rate. However, unlike the normal assumption, plantations exhibited lower SOC and TN stocks due to poor undergrowth and overexploitation for charcoal and firewood production including the roots. Specifically, among the three watersheds, Aba Gerima was found under a severely degraded situation owing to prolonged cultivation and unsustainable human activities, thus revealing the need for immediate land management interventions.

Chapter 3 clarifies the microbial potential of soils from Church Forest in Aba Gerima and its effect on seedling establishment of *Olea europaea* and *Albizia gummifera* native tree species under glasshouse conditions. The results showed significantly higher plant height, root collar diameter, shoot and total biomass for seedlings grown in non-sterilized (with microbes) forest soils than those grown in sterilized (without microbes) soil. Furthermore, the relative abundance of *Acidobacteria, Actinobacteria, and Nitrospirae* was significantly correlated with non-sterilized forest soil bacterial community ($r^2 = 0.6–0.8$, $p < 0.001$). The favorable soil pH environment of the forest soils was found to affect the abundance of the bacterial community in the Church Forest soils. Overall, seedlings grown in Church Forest soils showed better performance and survival rate, because of higher soil microbial abundance and diversity, than those grown in the soil from degraded land. This effect was more noticeable on the performance of *Olea* seedlings grown in the soil from *Croton macrostachyus*. This suggests that soil from remnant Church Forests, particularly soil from beneath *Croton macrostachyus*, can serve as a good inoculum source for native tree seedling growth and survival in degraded lands.

Chapter 4 evaluated the role of Church Forest soil in assisting seedlings of *Olea europaea* establishment under a degraded land condition in Aba Gerima watershed. Plots assisted with intact Church Forest (n=3) soil and without (n=3) were arranged in randomized block design with three replicates. In each plot, 16– *Olea* seedlings were planted in 1m x 1m spacing in July 2018. Monitoring for growth-related parameters was conducted on a monthly time basis. The results showed that *Olea* seedlings assisted with soil from Church Forest had higher growth and survival than seedlings without Church Forest soil. The height of assisted seedlings with a mean value of 85.7 (±5.3) cm compared to 74.5 (±2.3) cm in non-assisted ones. Significantly higher mortality of non-assisted seedlings was observed particularly during the months of 3–6 starting from the planting date. The survival was 89% and 77% for assisted and non-assisted seedlings, respectively. The results of this study confirm that Church Forest soils can assist seedlings establishment of native tree species in degraded lands.

Chapter 5 provides a general synthesis of the whole thesis based on the key findings of Chapters 2-4. These findings confirm that Church Forest soils are endowed with beneficial native microbes (mainly bacteria and fungi), which can serve as a source for native soil inoculum. Use of this inoculum source can facilitate native trees seedlings establishment, growth and survival in degraded lands restoration.

学位論文概要

土地の劣化は、2015 年の「世界の土壌資源の現状」報告書で世界的に最大の懸念事項と特定されている。エチオピアでは、その長期にわたる持続不可能な農業の歴史のため、土地の劣化が農業生産やその他の生態系サービスに最も深刻な問題を引き起こしている。一方で、その対策として、各地で植林や（人や動物の立ち入りを防ぐための）禁牧柵などの取り組みが行われている。このような取り組みにより、土壌の肥沃度や植生被覆は改善されたものの、在来樹種を植えることで生態系の機能や多様性を回復させることは、そのプロセスの遅さや、劣化した土地に生息する有益な土壌微生物の利用率の低さから、困難な課題であることがわかった。「教会の森 (Church Forests)」と呼ばれる小さな自然林は、エチオピアの原生林の遺された植生であり、1 世紀以上にわたって保護されてきた。教会林は、その社会的・精神的価値に加えて、生物多様性の中心地であり、在来の動植物の種子源や遺伝子の保全地としても利用されている。

しかし、土壌有機炭素（SOC）や全窒素（TN）など、土地の劣化や植林の主要な生態学的指標に関する科学的情報は、根拠に基づいた土地管理の導入を優先させ、設計するためには乏しい。さらに、微生物群集の役割を特徴づける研究や、劣化した土地での在来樹木の実生定着段階における教会の森の微生物の接種可能性や効果を評価する研究も不足している。

そこで、本研究の全体的な目的は、教会の森の土壌を在来微生物の供給源として利用することで、劣化した土地の在来樹木の再生を支援することに貢献することである。具体的な目的は以下の通りである。①青ナイル川上流域の 3 つの農業生態系で見られる異なる土地利用と地形における SOC と TN の含有量を評価することで、優先的に管理を導入すべき深刻な劣化地域を特定すること、②実験温室条件下

での教会の森土壌の土壌微生物学的潜在能力と選択された在来樹種の実生定着に及ぼす影響を明らかにすること、③劣化した土地の実験圃場条件下で選択された在来樹種の実生定着を促進する上での教会の森土壌の役割を評価することである。本論文は以下のように5つの章から構成されている。

第 1 章では、研究の導入部を説明する。研究の背景、問題点、目的、研究地域の説明を示し、さらに、文献調査に基づいて、土地の劣化と森林破壊、生態学的修復の取り組み、劣化した土地での実生定着の概要を説明している。その上で、本研究の目的と論文の全体構成について述べる。

第 2 章では、青ナイル川上流域の3つの農業生態系における3つの選択された流域の土地利用、地形的位置におけるSOCとTNの含有量を示す。調査対象となった流域は、Guder、Aba Gerima、Dibatieであり、それぞれ青ナイル川上流域の高地、中地、低地の農業生態系を表している。3つの流域の4つの土地利用、3つの地形、3つの土壌深さから採取した合計352個の土壌サンプルを用いて、SOCとTNを分析した。その結果、土地利用、地形、農耕生態系、それらの相互作用が両者の含有量に大きな影響を与えていることがわかった。その結果、耕地利用が最も劣化しているのは上部地形に位置する農地であることが明らかになった。しかし、想定とは異なり、植林地では下草の生育が悪く、根を含めた炭や薪の生産のために過剰に利用されているため、SOCとTNの含有量が低くなっていることが明らかになった。具体的には、3つの流域のうち、Aba Gerimaは、長期にわたる耕作と持続不可能な人間活動により深刻な劣化状態にあり、早急な土地管理の導入が必要であることが明らかになった。

第 3 章では、Aba Gerima の教会の森の土壌の微生物ポテンシャルと、実験温室条件下での *Olea europaea* と *Albizia gummifera* の在来樹種の実生定着に及ぼす影響を明らかにした。その結果、殺菌処理されていない（微生物を含む）森林土壌で栽培された実生苗は、殺菌処理されていない（微生物を

含まない）土壌で栽培された実生苗と比較して、植物高、根径、茎部バイオマス、総バイオマスが有意に高いことが示された。さらに、*Acidobacteria*、*Actinobacteria*、*Nitrospirae* の相対的な豊富さは、非滅菌森林土壌の細菌群集と有意な相関があった（$r^2 = 0.6〜0.8$, $p < 0.001$）。森林土壌の良好な土壌 pH 環境は、教会の森土壌の細菌群集の豊富さに影響を与えることがわかった。全体的に、教会の森の土壌で育った苗は、より良い性能と生存率を示した。この影響は、*Croton macrostachyus* の土壌で栽培された *Olea* の苗の性能に顕著であった。このことは、教会林の土壌、特に *Croton macrostachyus* の下の土壌が、荒廃した土地での在来樹の苗の生育と生存に適した接種源となりうることを示唆している。

第 4 章では、Aba Gerima 流域の荒廃した土地条件の下で、*Olea europaea* の苗木の定着を支援するための教会林の土壌の役割を評価した。教会の森の土壌を用いた圃場（n=3）と用いなかった圃場（n=3）を、乱塊法で 3 つの繰り返し処理を用いて配置した。各プロットでは、2018 年 7 月に 16 の *Olea* 苗を 1m×1m の間隔で植え付けた。成長に関連するパラメータを毎月の時間ベースで測定した。その結果、教会の森から採取された土壌を用いて育成された *Olea* の苗（補助苗）は、教会の森土壌を育成に用いない苗（非補助苗）よりも高い成長と生存率を有していた。植物高の平均値は、非補助苗の 74.5（±2.3）cm に対し、補助苗で 85.7（±5.3）cm であった。非補助苗は、特に定植日から 3〜6 ヶ月の間に有意に高かった。生存率は、補助苗で 89%、非補助苗で 77%であった。本研究の結果は、教会林の土壌が荒廃地における在来種の実生定着を促進することを確認した。

第 5 章では、第 2 章から第 4 章で得られた重要な知見に基づいて、論文全体を総合的にまとめている。本研究の結果から、教会の森の土壌には有益な在来微生物（主に細菌と菌類）が生息しており、これが在来土壌の接種源となり得ることが示された。この接種源を利用することで、劣化した土地の修復にお

いて、在来樹木の実生の定着、成長、生存を促進することができる。

List of Publications

1. Abebe, G., Tsunekawa, A., Haregeweyn, N., Takeshi, T., Wondie, M., Adgo, E., Masunaga, T., Tsubo, M., Ebabu, K., Berihun, M. L. & Tassew, A. 2020. Effects of Land Use and Topographic Position on Soil Organic Carbon and Total Nitrogen Stocks in Different Agro-Ecosystems of the Upper Blue Nile Basin. *Sustainability,* 12**,** 2425 (Published, https://doi.org/10.3390/su12062425, this article covers Chapter 2 in the thesis).

2. Abebe, G., Tsunekawa, A., Haregeweyn, N., Taniguchi, T., Wondie, M., Adgo, E., Masunaga, T., Tsubo, M., Ebabu, K., Mamedov, A., and Meshesha, D.T. 2020. Effect of Soil Microbiome from Church Forest in the Northwest Ethiopian Highlands on the Growth of *Olea europaea* and *Albizia gummifera* Seedlings under Glasshouse Conditions. Sustainability, 12, 4976 (Published, https://doi.org/10.3390/su12124976, this article covers Chapter 3 in the thesis).

Appendix

Table S1. SOC and TN stocks at three soil depths under different land-use types at Guder, Aba Gerima, and Dibatie

	SOC stock (Mg ha–1)			TN stock (Mg ha–1)		
Land use	**Guder**	**Aba Gerima**	**Dibatie**	**Guder**	**Aba Gerima**	**Dibatie**
Soil depth 0–15 cm						
Bushland	47.23 (1.09) ^{a}	23.26 (4.75) ^{aA}	30.22 (3.74) ^{aA}	4.16 (0.09) ^{a}	1.68 (0.36) ^{a}	4.86 (0.92) ^{aA}
Cropland	21.39 (0.81) ^{bc}	9.74 (0.76) ^{bA}	29.05 (3.05) ^{aA}	1.65 (0.06) ^{bc}	0.72 (0.09) ^{b}	3.76 (0.72) ^{abA}
Grazing land	34.80 (1.64) ^{ab}	19.83 (1.40) ^{aA}	29.05 (2.94) ^{aA}	2.80 (0.14) ^{ab}	1.74 (0.11) ^{a}	3.02 (0.64) ^{bA}
Plantation	15.05 (0.62) ^{c}	20.96 (2.88) ^{aA}	na	1.19 (0.04) ^{c}	1.50 (0.24) ^{a}	na
Soil depth 15–30 cm						
Bushland	43.47 (0.82) ^{a}	15.13 (2.30) ^{aA}	25.88 (3.73) ^{aA}	3.63 (0.07) ^{a}	1.73 (0.24) ^{a}	1.56 (0.19) ^{aB}
Cropland	19.68 (0.79) ^{b}	8.11 (0.76) ^{bA}	21.18 (3.05) ^{aAB}	1.53 (0.05) ^{b}	0.66 (0.08) ^{b}	1.28 (0.13) ^{bB}
Grazing land	33.52 (1.43) ^{a}	13.07 (1.20) ^{aAB}	20.78 (3.05) ^{aA}	2.66 (0.11) ^{a}	1.16 (0.09) ^{ab}	1.26 (0.17) ^{bB}
Plantation	15.61 (0.74) ^{b}	13.80 (2.11) ^{aB}	na	1.23 (0.04) ^{b}	1.07 (0.18) ^{ab}	na
Soil depth 30–50 cm						
Bushland	50.49 (0.81) ^{a}	19.48 (4.15) ^{aA}	27.68 (3.74) ^{aA}	4.11 (0.08) ^{a}	1.42 (0.35) ^{a}	1.67 (0.21) ^{aB}
Cropland	19.92 (0.98) ^{b}	8.12 (1.02) ^{bA}	22.39 (3.05) ^{aB}	1.59 (0.07) ^{b}	0.75 (0.13) ^{a}	1.22 (0.10) ^{bB}
Grazing land	41.62 (1.89) ^{a}	11.24 (1.63) ^{abB}	22.69 (3.18) ^{aA}	3.22 (0.13) ^{a}	1.27 (0.17) ^{a}	1.24 (0.11) ^{abB}
Plantation	18.13 (0.82) ^{b}	11.75 (2.04) ^{abB}	na	1.44 (0.05) ^{b}	1.12 (0.22) ^{a}	na

Different lowercase letters indicate significant differences among land uses at each soil depth; different capital letters indicate significant differences among soil depths within the same land-use type (mean ± SE; Tukey HSD, $P < 0.05$, $n = 12$). na: not applicable.

Table S2. SOC and TN contents and stocks at the three topographic positions of the study sites

Land use	**Site**	**SOC**		**TN**		**C/N ratio**	**Bulk density**
		mg g–1	**Mg ha–1**	**mg g–1**	**Mg ha–1**		**Mg m–3**
Bushland	Guder	31.63 (1.45)a	141.19 (6.74)a	2.63 (0.13)a	11.73 (0.50)a	12.16 (0.30)a	0.90 (0.01)b
	Aba Gerima	13.42 (1.16)b	59.23 (7.19)b	0.96 (0.09)b	4.13 (1.91)b	14.01 (0.31)a	1.09 (0.03)a
	Dibatie	15.31 (1.19)b	85.70 (9.81)ab	0.92 (0.06)b	8.08 (1.51)a	16.16 (0.57)a	1.11 (0.04)a
Cropland	Guder	13.07 (1.04)a	61.00 (2.33)a	1.02 (0.07)a	4.77 (0.28)b	12.52 (0.32)b	0.96 (0.03)b
	Aba Gerima	5.01 (0.29)b	25.97 (7.04)b	0.39 (0.03)b	2.14 (1.44)c	17.63 (2.33)a	1.12 (0.01)a
	Dibatie	13.28 (0.96)a	72.62 (8.33)a	0.75 (0.04)a	6.26 (1.34)a	17.38 (0.82)a	1.12 (0.03)a
Grazing	Guder	18.19 (1.20)a	109.94 (3.69)a	1.44 (0.09)a	8.68 (0.31)a	12.68 (0.20)b	1.18 (0.04)b
	Aba Gerima	7.97 (0.49)b	44.14 (7.55)b	0.71 (0.04)b	4.17 (1.19)b	11.03 (0.17)b	1.19 (0.03)b
	Dibatie	10.84 (0.81)b	66.42 (4.69)b	0.63 (0.05)b	5.65 (1.52)ab	17.52 (0.72)a	1.32 (0.02)a
Plantation	Guder	9.85 (0.87)a	48.79 (7.15)a	0.78 (0.06)a	3.86 (0.65)a	12.16 (0.41)b	1.02 (0.02)a
	Aba Gerima	9.24 (0.79)a	44.77 (6.33)a	0.68 (0.06)a	3.56 (0.38)a	17.15 (2.62)a	1.06 (0.03)a

Different letters within each column indicate a significant difference between sites for each topographic position at $P < 0.05$.

Values are mean (SE) across the entire soil depth from 0 to 50 cm.

Table S3. ANOVA of *Olea*, *Albizia* and both seedlings showing results for plant height (H), root collar diameter (RCD), survival rate (SR), shoot mass (SB), root mass (RB), root to shoot ratio (R/S), total mass (TB), soil pH (pH), soil organic carbon (SOC), total nitrogen (TN), carbon to nitrogen ratio (C/N), and available phosphorus (P).

Species	**Source of variation**	**df**	**P-value**											
			H	**RCD**	**SR***	**SB**	**RB**	**R/S**	**TB**	**pH**	**SOC**	**TN**	**C/N**	**P**
Olea	Trt	1	<0.0001	<0.0001	0.061	<0.0001	0.0001	<0.0001	<0.0001	0.2629	0.350	0.245	0.712	0.050
	IS	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.658	0.138	0.684	<0.0001
	Trt × IS	3	<0.0001	0.002	0.062	<0.0001	0.0079	<0.0001	0.0007	0.0006	0.600	0.174	0.166	0.610
	Error	55												
Albizia	Trt	1	<0.0001	0.002	0.042	<0.0001	0.990	<0.0001	0.007	<0.0001	0.5731	0.512	<0.0001	<0.0001
	IS	3	<0.0001	<0.0001	0.008	<0.0001	<0.0001	0.116	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Trt × IS	3	0.005	0.002	0.419	<0.0001	<0.0001	0.605	<0.0001	0.013	0.069	0.317	<0.0001	0.0005
	Error	55												
Overall	Sp	1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7897	<0.0001	0.0176	0.0652	0.0484	0.0347	0.0591
	IS	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0437	<0.0001	<0.0001	0.1985	<0.0001
	Trt	1	<0.0001	0.0038	0.0056	<0.0001	0.3051	<0.0001	<0.0001	0.1902	0.0900	0.0942	0.1394	0.0165
	Sp × IS	3	0.0023	0.0366	<0.0001	0.0013	0.0013	0.5427	<0.0001	0.2630	<0.0001	<0.0001	0.1596	0.7048
	Sp × Trt	1	0.1043	0.1705	<0.0001	0.0219	0.0765	0.2866	0.0231	0.8691	0.3850	0.3070	0.9740	0.0331
	Trt × IS	3	<0.0001	0.0015	<0.0001	<0.0001	<0.0001	0.3592	<0.0001	0.1545	0.0492	0.0578	0.0998	0.8996
	Sp × IS × Trt	3	0.0193	0.0101	<0.0001	<0.0001	<0.0001	0.8371	<0.0001	0.2368	0.0311	0.0232	0.4101	0.1402
	Error	112												

Species (Sp) are *Olea and Albizia* seedlings, soil treatment (Trt) are seedlings with (non-sterilized) and without (sterilized) soil and four different soil origins including degraded land (DL), or soil from beneath *Albizia gummifera* (AG), *Croton macrostachyus* (CM), and *Juniperus procera* (JP) as soil origin (IS). * Log-rank test statistics for main effects (Trt and IS) and cox-regression analysis for interaction effect (Trt × IS, Sp × IS, Sp × Trt and Sp × IS × Trt)

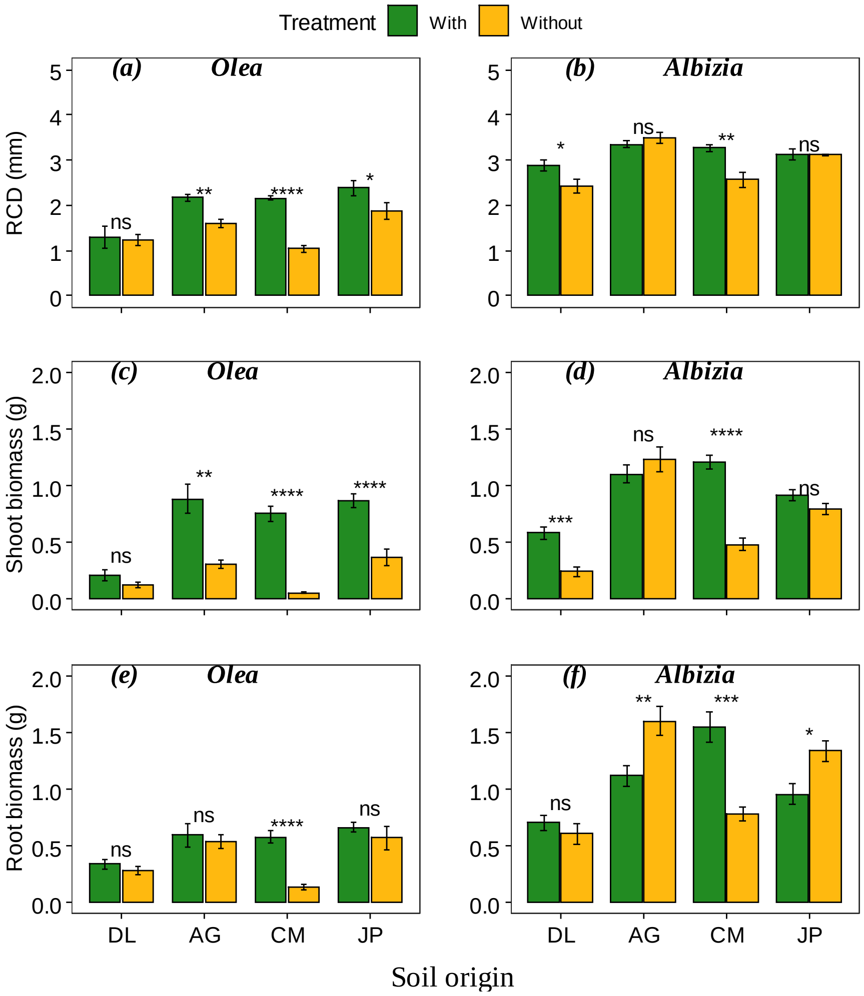

Figure S1. Effects of soil from different origins on root collar diameter (RCD) (**a, b**), shoot biomass (**c, d**), and root biomass (**e, f**), in *Olea* and *Albizia* seedlings, respectively, with treatment (non-sterilized soil) and without treatment (sterilized soil). DL, AG, CM, and JP stand for soil origins from degraded land, or from beneath *Albizia gummifera*, *Croton macrostachyus*, *Juniperus procera*, respectively. Asterisks indicate statistically significant differences between seedlings with treatment (in non-sterilized soil) and without treatment (in sterilized soil): * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$; and ns, not significant). Values are mean ± standard error (n = 8)