Genetic and Physiological Diversities of Bacteria from the Rhizosphere of Woody Plants Grown on Soil from Girar Jarso District and their Effect on the Growth and Establishment of Tree Seedlings in North Shewa Zone, Ethiopia

By

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This is to certify that the Dissertation prepared by **Alemayehu Getahun**, entitled: "**Genetic and Physiological Diversities of Bacteria from the Rhizosphere of Woody Plants Grown on Soil from Girar Jarso District and their Effect on the Growth and Establishment of Tree Seedlings in North Shewa Zone, Ethiopia**" and submitted in fulfilment of the requirement for the Degree of philosophy (Applied Microbiology) complies with the regulations of the University and meet the accepted standards with respect to originality and quality.

Supervisors:

Diriba Muleta (PhD, Associate Prof., AAU) Signature _____________

Fassil Assefa (PhD, Associate Prof., AAU) Signature ______________

April 2021

Dedication

The work is dedicated to my beloved uncle Wudineh Kassa, he is the reason for my presence here today. He passed a lot of ups and downs for me and he is my hero and my lifesaver who cherished and nurtured seedling of my education and without whose love, blessing, inspiration, excellent support and encouragement I would not come this far.

Declaration

This is to declare that the whole of the research work reported here in the dissertation entitled **“Genetic and Physiological Diversities of Bacteria from the Rhizosphere of Woody Plants Grown on Soil from Girar Jarso District and their Effect on the Growth and Establishment of Tree Seedlings in North Shewa Zone, Ethiopia”** for the fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Microbial, Cellular and Molecular Biology (Applied Microbiology) to the School of Graduate Studies of Addis Ababa University is my own independent work and has not been previously submitted by me (PhD candidate) or anybody elsewhere for any other degree so far. The materials obtained from other sources have been dually acknowledged in the dissertation.

Alemayehu Getahun Kassa

April, 2021

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ABSTRACT

Land degradation (LD) is one of the major problems the planet earth has been facing. It has severely affected 1.9 billion hectares and decreased ecosystem services by 60%. In Eastern Africa, particularly in the northern highlands of Ethiopia, 1 billion tons of topsoil are lost annually. Thus, the land becomes bare, nutrient-depleted, water-stressed and abandoned by the community. Hence, it needs urgent rehabilitation with the application of eco-friendly microbes and organic amendments (OAs) through the exploitation of different types of trees/shrubs. Therefore, the overall objective of the study was to rehabilitate degraded habitat through the application of OAs and phytobeneficial bacteria for the establishment of multipurpose trees. Soil samples were collected from nine random corners at depth of 30 cm for soil physicochemical analysis before and after OAs. Six different treatments (biochar, compost, manure, mixed, bacterial inoculation (BI), and control) were considered at a 1:1 ratio of OAs per pot. Application BI was done for field trials during transplanting and quarterly for a year. Six plots measuring 41 m x 4 m were established in completely randomized block design and assigned at the random block for the field trial. Following OAs, microbial counts were done for one year every month. Bacteria were isolated from the rhizosphere of Acacia and Juniperus. The primary selection of isolates was based on drought stress (DS) tolerance and phosphate solubilization, other stress, and plant growth-promoting traits. The potential isolates were subjected to carbohydrate and amino acid utilization tests, BOX PCR, and 16S rRNA profiling. DS tolerant, phosphate solubilizer strains with multiple plant growth-promoting traits were chosen for Acacia seeds germination and field application alone and in combination with OAs. Plant growth parameters and their survival rate at each amended plot were assessed. There is a significant increase in soil pH (5.69-8.13), CEC (43.78-49.98 $\mathrm{cmol_c/kg}$), OM (2.43-3.91%), total nitrogen (0.13-0.76%), and available P (18.9-26.31 ppm) following OAs compared the control. Combined treatment had the largest effect on cover crops biomass with 3.43 g, 4.54 g, 0.7 g, 2.07g in alfalfa, grass pea, and control respectively $p \leq 0.05$. The C and N utilization revealed metabolic versatility of the strains (14.29 to 100%). *Ochrobactrum*, *Pseudomonas*, and *Klebsiella* spp expressed remarkable metabolic versatilities. BOX-PCR showed greater genetic diversity and confirmed by Simpson's Index of Diversity (0.883) took the leading position with *Bacillus* species. The 16S rRNA genes sequence showed 21.92% Firmicutes and 78.08% Proteobacteria with *Pseudomonas* 23% and *Ochrobactrum* 21% dominant species. Out of 73 isolates, 10 (14%) were highly tolerant of 40% polyethylene glycol. All the isolates can grow in wider ranges of pH (5-9), temperature (15-45°C). The inoculated bacterial strains significantly $p \leq 0.05$ increased root, shoot length, and dry biomass of acacia. According to solubilization index (SI) 45% isolates were classified as high and medium phosphate solubilizers with 195 to 373 µg/mL. The maximum P and IAA were produced by *Pseudomonas* FB-49 (373 and 659.07µg/mL), respectively. The highest (100%) seed germination caused by *Pseudomonas* BS-26 and *Pseudomonas* FB-49. There is a significant difference in microbial counts following OAs compared to the control. The greatest counts in bacteria, actinomycetes, and fungal (21.66, 2.29, 0.82 x 10^5 CFU g^{-1}) of soil, respectively in the combined amended plot. There was a significant increase in stem height, girths, and branch numbers in amended plots relative to the control. The survival rate was observed in apple (80%) followed by acacia (66%) and prunus (51%). The survival is in the order of BI x OAs > BI > biochar > compost > manure > control amended soil. This study concluded that degraded land could be rehabilitated with cheap OAs, potential bacterial strains, and bring multipurpose tree establishment with greater survival rate and best performance.

Keywords: Acacia, DS, Ethiopia, Multipurpose trees, OAs, Plant growth-promoting rhizobacteria, Rehabilitation, Survival rate, Tree establishments

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List of Abbreviation

ACC	Aminocyclopropane-1- Carboxylate
AGDP	Agricultural Gross Domestic Product
AIA	Actinomycetes Isolation Agar
ANOVA	Analysis of Variance
BAS	Biochar Amended Soil
BCMAS	Biochar, Compost and Manure Amended Soil
BC	Biochar
BI	Bacterial Inoculations
BLAST	Basic Local Alignment Search Tools
CAS	Compost Amended Soil
CBD	Complete Block Design
CC	Climate Change
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
CMC	Carboxymethyl Cellulose
CRA	Congo red Agar
CTRL	Control
DMRT	Duncan’s Multiple Range Test
DS	Drought Stress

EC	Electrical Conductivity
EPS	Exopolysaccharide Production
FTC	Farmers Training Center
FYM	Farm Yard Manure
GHG	Greenhouse Gases
GPS	Global Positioning System
HCN	Hydrogen Cyanide
HM	Heavy Metals
HPS	High Phosphate Solubilizers
HT	Highly Tolerant
IAA	Indole Acetic Acid
LD	Land Degradation
LS	Low Solubilizers
MAS	Manure Amended soil
MEGA	Molecular Evolutionary Genetics Analysis
MPS	Medium Phosphate Solubilizer
MSM	Minimal Salt media
NCBI	National Center for Biotechnology Information
NDW	Nodule Dry Weight
NFW	Nodule Fresh Weight

NN	Nodule Number
OA	Organic Amendments
OD	Optical Density
OM	Organic Matter
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PGPR	Plant Growth Promoting Rhizobacteria
PM	Plate Method
PSI	Phosphate Solubilization Index
RDW	Root Dry Weight
RFW	Root Fresh Weight
RL	Root Length
SAS	Statistical Analysis System
SD	Standard Deviation
SDW	Shoot Dry Weight
SFW	Shoot Fresh Weight
SH	Shoot Height
SI	Solubilization Index
SOC	Soil Organic Carbon

TCP	Tricalcium Phosphate
TM	Tube Method
TN	Total Nitrogen
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
VI	Vigor Index
VLS	Very Low Solubilization

Table of Contents

List of Tables

List of Figures

List of Appendices

Publications

This thesis is based on the work contained in the following papers:

1. **Alemayehu Getahun**., Solomon Kiros., Diriba Muleta, and Fassil Assefa, (2020a). Genetic and metabolic diversities of rhizobacteria isolated from degraded soil of Ethiopia. *Heliyon*, **6,** 1-10.
2. **Alemayehu Getahun**., Diriba Muleta., Fassil Assefa., Solomon Kiros, and Hungria, M. (2020b). Biochar and Other Organic Amendments Improve the Physicochemical Properties of Soil in Highly Degraded Habitat. *Eur. J. Eng. Res. Sci.*, **5,** 331-338.
3. **Alemayehu Getahun**., Diriba Muleta., Fassil Assefa., Solomon Kiros. (2020c). Plant Growth-Promoting Rhizobacteria Isolated from Degraded Habitat Enhance Drought Tolerance of Acacia (Acacia abyssinica Hochst. ex Benth.) Seedlings. International Journal of Microbiology., **2020,** 1-13.
4. **Alemayehu Getahun**., Solomon Kiros., Diriba Muleta, and Fassil Assefa, (2020d). Phytobeneficial Traits of Rhizobacteria Isolated from Degraded Soil and their Effect on improving Seeds Germination of Acacia (Acacia abyssinica Hochst. ex Benth). Journal of Applied Biology and Biotechnology. **Accepted**
5. **Alemayehu Getahun**., Solomon Kiros., Diriba Muleta, and Fassil Assefa, (2020e). The effect of Bacterial Inoculation and Organic Amendment for Establishment of Some Multipurpose Trees on Degraded Land. **Ready for submission on Journal of Environmental management.**

Chapter 1

1. General Introduction

Franklin D. Roosevelt, the President of the USA in 1937 coined the famous phrase “When soils fail, civilizations fall”, while he was addressing the shock of the “American Dust Bowl” that had destroyed millions of hectares of arable land in the Midwest of the USA. This phenomenon is still relevant in the tropics which is a reminder to protect soil which is a valuable base for ecosystem services and production (Schröder *et al.*, 2017).

The world is currently in a period of rapid, anthropogenic driven environmental changes brought about by land cover changes, fragmentation, invasive species, and pollution (Montoya-Tangarife *et al.*, 2017). Fragmentation of natural systems occurs whenever removal of pre-existing land cover and replacement of other cover types occur, be it urban, agriculture, forestry production or other land uses (Schwartz and van Mantgem, 1997). This fragmentation of natural systems leads to land degradation (LD) which is a global long-term loss of ecosystem function and productivity due to natural and human-induced disturbances and is a threat to the ecological, economic, and quality of the land of the planet earth (Giuliani *et al.*, 2020; Naseer and Pandey, 2018).

Nearly 1.9 billion hectares of land are affected and 1.5 billion people are threatened worldwide by LD (Low, 2013). As a result, LD decreased soil ecosystem services by 60% between 1950 and 2010 León and Osorio (2014), and brought the global loss of about 5-8 million hectares of productive land annually out of cultivation (Kirui and Mirzabaev, 2016). Land-use changes in East Africa have transformed natural land covers to farmlands, grazing lands, human settlements, and urban centers at the expense of natural vegetation and loss of biodiversity (Maitima *et al.*, 2009).

Land degradation in Ethiopia is the main threat in drylands which covered about 75 million hectares (ha) of land EARO (2000), due to agricultural intensification without soil and water conservation leading to unsustainable crop production (Tewolde BG Egziabher, 1989). Messay Tefera (2011) also estimated that the highland of Ethiopia covered 45% of the country and is a habitat of more than 88% of the population, and suffered from overpopulation, extensive cultivation of even stiff slopes with croplands resulting infrequent incision by ravines and formation of gullies. It is estimated that nearly1 billion tons of topsoil are lost annually in Ethiopia due to soil erosion costing 3% of its Agricultural Gross Domestic Product (AGDP) (Yesuf *et al.*, 2008). This, together with climate change and population growth, could lead to a further decline in environmental goods and services, including loss of biodiversity and soil fertility.

Land degradation has been identified as the most serious environmental problem in the Ethiopian highlands (lying above 1,500 m above sea level) that comprise nearly 44% of the country's total area and about 88% of the human population (Amare Haileslassie *et al.*, 2005). Currently, these areas are typified by the degradation of vegetation cover and widespread soil degradation. The conversion of forests, woodlands, and shrublands into croplands and cultivation of steep slopes by traditional ox plow technology have resulted in the loss of natural vegetation cover and severe soil erosion (Aune *et al.*, 2001; Tagel Gebrehiwot and Veen, 2014). According to the World Bank (2001) report, the rate of deforestation in the highlands is estimated to be 150,000 ha per year.

The continuous LD can be reversed through ecological restoration as the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (Martin, 2017). It is an important strategy to reverse LD, rehabilitate landscape integrity, and realize the environmental and socioeconomic benefits of natural resources (Hobbs and Cramer, 2008). Areas undergoing restoration processes can also perform food and nutritional security, income generation, conservation of water resources, balance in climate and biodiversity, among others. To reverse LD, several rehabilitation measures are recommended. These include establishing plantation forests via afforestation and reforestation Kanninen (2010), closing areas for rehabilitation Daniel Mengistu *et al.* (2016), promotion of agroforestry systems Sánchez *et al.* (1994) and establishing nurseries for the production of a diverse range of tree and shrub species to enhance soil fertility (Haase and Davis, 2017).

Area closures (enclosures) are one of the managements of rehabilitating degraded lands that have been excluded from human and livestock interference and left to regenerate naturally (Daniel Mengistu *et al.*, 2016; Kiros Abay *et al.*, 2020). Area closures aim to prevent further degradation of the ecosystems, advance re-vegetation, and restore the overall ecological conditions of the area. There are two types of closures 1) only closing the area from interferences of human interventions (leaving it to natural regeneration) and 2) closing off degraded land while simultaneously implementing additional measures such as planting of tree seedlings, mulching, and establishing water harvesting structures to enhance and speed up the regeneration process.

In Ethiopia, enclosures are suggested to be important methods for rehabilitation and management of degraded land and its actual and potential socioeconomic profits in various parts of Ethiopia such as North Shewa (Mengistu Asmamaw, 2011), Rift valley (Daniel Mengistu *et al.*, 2016; Mohammed Kasim *et al.*, 2015), highlands northern Ethiopia (Emiru Birhane *et al.*, 2007).

The other option of reversing current trends in land degradation is restoration processes by planting (afforestation) and land coverage with vegetation (green manure) Nunes *et al.* (2012) and agroforestry practices. To this end, there is a strong activity worldwide to cover 4.5 million ha of land with plants annually of which 91% was undertaken in Asia and South America (Nobre *et al.*, 2016). Although plantations are expanding, in Africa, they are moving at a slower pace (Clewell *et al.*, 2004). Agroforestry is a collective name for land-use systems and technologies where woody perennials are deliberately used on the same land-management units as crops and/ or animals, in either a spatial arrangement or a temporal sequence (Erdmann, 2005). Agroforestry systems can restore important ecological processes and enabling economic returns and strengthening livelihoods.

The establishment of plants in soils in general, and that of degraded land in particular, requires the tripartite interaction amongst the soil, the plant, and the microbes in the terrestrial habitat. Under natural systems, this interaction is complex and dynamic and facilitates ecosystem functioning balancing the sustainability of production (synthesis) and nutrient recycling (consumption) (Taschen *et al.*, 2020).

Microbes play a very important role in ecosystem functioning. They are involved in catabolic reactions of organic substances, energy flow, and nutrient recycling (Singh and Gupta, 2018). In the geochemical cycles carbon, nitrogen, sulfur, and phosphorus are important for building up living things. This, together with energy flow in the ecosystem is mainly facilitated by microorganisms (Madsen, 2011). For this reason, microorganisms are known as the "wastebasket" of the terrestrial and aquatic ecosystem. The activities that involve microbes that are currently termed as "ecosystem processes"- are the interactions among the biotic and abiotic elements Lyons *et al.* (2005), "ecosystem functioning"- Some key ecological processes, such as energy transfer, nutrient cycles, and oxygen and water fluxes Wallace (2007) are always regarded as synonymous with ecosystem function" and "ecosystem services" - the benefits that humans obtain from ecosystems or enhance social welfare Bennett *et al.* (2009) are implicated with energy flow and nutrient cycling that maintain sustainable production of the global ecosystem (Chagnon *et al.*, 2015).

Plants harbor different microorganisms as endophytes and rhizosphere microorganisms around their roots. They release up to 30% of their daily photosynthetic products to the soil to attract as many diverse microorganisms around the rhizosphere and rhizoplane (Schillaci *et al.*, 2019). Introducing native microbes to the soil speed up the microbial community to undergoing physicochemical changes induced by organic amendments (OAs) (van de Voorde *et al.*, 2012). There is increasing interest in amending degraded soils to improve soil quality, rehabilitation to enhance tree growth, and provision of ecosystem services (Wiseman *et al.*, 2012). Organic amendments, particularly compost, manure, and biochar are receiving renewed attention in restoring disturbed soils.

Plants and their associated microorganisms play a crucial role in the formation or modification of soil (Pate and Verboom, 2009). Rehabilitation of degraded habitat requires designing a plant succession that will give adequate surface cover and increase the fertility of the soil. Rapidly growing grasses to give ground cover as quickly as possible and stabilizes the surface; legumes to fix nitrogen and other grasses and shrubs to provide long-term cover (Tansey *et al.*, 2008). Soil microbial communities are recognized as below-ground ecosystem engineers and directly influence the above-ground plant community (Kardol and Wardle, 2010). Microbial inoculations help to establish diverse and functional microbial communities in soils and improve plant growth (De-Bashan *et al.*, 2010b).

Inoculation of plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi (MF) are beneficial to root nutrient acquisition and plant tolerance of stresses (Alguacil *et al.*, 2011). There has been a rebirth of interest in environmentally friendly and sustainable practices (Esitken *et al.*, 2005). The use of PGPR as biofertilizers are promising biotechnology to improve productivity with cheap and eco-friendly inputs Bashan (1998), via nutrient mobilization, biocontrol, phytohormone production, and nitrogen fixation (Glick, 1995). Among practices available for reforestation for timber production, inoculation with PGPR and AMF is attained acceptance (Bashan *et al.*, 2012).

The inoculation of beneficial soil community may not be enough to assure the establishment of plant cover. Moreover, *in vitro* studies reported by Diriba Muleta (2007) mentioned *Bacillus*, *Erwinia*, *Ochrobactrum*, *Pseudomonas*, and *Serratia* spp. isolated from the coffee rhizosphere as the most important isolates to act as potential biofertilizers, biocontrol agents, or both. Recently, inoculation with PGPR and AMF was demonstrated in the screenhouse cultivation of four Sonoran Desert native trees (Bashan *et al.*, 2009).

For successful rehabilitation, it is necessary to improve soil quality and the ability of the plant species to resist harsh environments through OAs (Medina *et al.*, 2004). The beneficial effects of OAs include the provision of plant nutrients, increased water holding capacity, improved soil structure, and increased microbiology activity (Caravaca *et al.*, 2002). It is one of the options to rehabilitate soil organic matter and associated soil structure and functions in degraded soils (Huang *et al.*, 2012). The most commonly used OAs are crop residues and their compost, manure, un-composted organic materials, such as sludge, plant residues, and biochar (Medina and Azcón, 2010).

Combining microbial inoculation with OAs greatly enhances the success rate and microbiological activity. Fast-growing legumes and grasses are considered pioneer crops for early vegetation purposes and needed to stabilize soils. They can offer superior tolerance to drought, low soil nutrients, and other climatic stresses. The initial cover must allow the development of diverse self-sustaining plant communities (Ranjan *et al.*, 2015). Sometimes multipurpose trees are considered in restoring the degraded lands besides improving soil fertility (Assel, 2006). Trees are very efficient in biomass generation by adding more organic matter to the soil, both above and below ground than other plants (Mertens *et al.*, 2007). A mix of plant species is required because it is impossible to predict the success of any one species in marginal environments where vegetation is going to receive little or no maintenance (Tansey *et al.*, 2008).

The selection of the most efficient beneficial rhizobacteria and the combined effect with OAs is a critical point for rehabilitation of degraded soils. Their role in nutrient recycling is priceless to the development and stability of an ecosystem (Clewell and Aronson, 2013). The use of OAs in the ecosystem accelerates soil regeneration processes in degraded lands. This recovery is a result of the rapid increment of organic matter contents in the soil in the short term (Hueso-González *et al.*, 2017).

PGPR confer beneficial effects through two different ways: indirectly or directly (Glick, 1995; Kloepper *et al.*, 2004). Indirect promotion of plant growth occurs when these bacteria decrease some of the deleterious effects of a phytopathogenic organism (often a fungus or a bacterium) by one or more different mechanisms. They also elicit physical and chemical changes in plants that result in enhanced tolerance to abiotic stress such as drought, salt, and nutrient deficiency and heavy metals with a process referred to as induced systemic resistance (ISR) (Glick, 2012; Van Loon, 2007).

The direct promotion of plant growth often includes the uptake of nutrients such as P and Fe from the environment. The bacteria often solubilize mineral (inorganic) P through the synthesis of low molecular weight organic acids such as gluconic and citric acid (Rodriguez *et al.*, 2004). They mineralize organic P through the synthesis of phosphatases (Rodríguez *et al.*, 2006). According to Glick *et al.* (2007b), PGPR produce phytohormone to promote plant growth in the presence of environmental stresses. PGPR fix atmospheric nitrogen, synthesize siderophores that can solubilize and sequester iron from the soil to provide to plants (Rodríguez and Fraga, 1999).

Several studies have proved that the effects of drought stress (DS) may be mitigated to a significant extent by the application of PGPR. Leyva and Bashan (2008) showed inoculation of *Azospirillum brasilense* Cd, into the legume plant mesquite amargo (*Prosopis articulate*) enhanced the growth of the seedlings taller and greener (13.14 ± 0.56 cm) than uninoculated plants (5.71 ± 0.34 cm) in the

Sonoran Desert of the USA. Co-inoculation of *R. tropici and P. polymyxa* showed a many-fold increase in the growth, nitrogen content, phytohormone levels, and nodulation of common bean (*Phaseolus vulgaris*) under three levels of drought compared with *Rhizobium* alone (Figueiredo *et al.*, 2008). *P. putida, Pseudomonas* sp., and *Bacillus megaterium,* isolated from drought affected soil also able to stimulate mung bean, *Vigna radiate* growth under dry conditions (Saravanakumar *et al.*, 2011).

The effect of OAs on physicochemical properties, growth, and activity of soil biota has not been sufficiently evaluated with field trials. If urgent measures are not taken, the country is headed for a "catastrophic situation" (Pender and Berhanu Gebremedhin, 2007). Hence, merging natural with human-made solutions will be needed to find a way to make our ecosystems compatible between nature and human use (Keesstra *et al.*, 2018). The efforts made in the past have rehabilitated degraded farmlands, improved soil water holding capacities, increased woodlots, and improved the productivity of the pastured lands in some watersheds in Ethiopia (Sonneveld and Keyzer, 2003). However, the current rate and status of environmental degradation still call for more extended and coordinated intervention actions (Edward, 2000; Kindeya Gebrehiwot, 2004).

In recent years, several microbial inoculants have been formulated, produced, marketed, and applied successfully by an increasing number of growers worldwide (Lucy *et al.*, 2004). However, these inoculants are not necessarily effective in all treatments that necessitate site-specific studies to fully realize their impact on the restoration of plants in degraded landscapes. Moreover, the effect of OAs on physicochemical properties and activity of soil biota has not been sufficiently evaluated with field trials. The role of soil microorganisms in rehabilitation of degraded habit is not yet appreciated as the main component of ecosystem particularly in Ethiopia. Thus, this work is its first type to move the application of potential rhizobacteria and OAs from agricultural practices to degraded land rehabilitation. Therefore, the main intention of the study is to assess the long-term benefits of inoculation, OAs and their combined effect on the establishment of key plant species in the rehabilitation of a representative degraded land.

1.1. Objectives of the study

General Objective

- The main objectives of the study are to characterize the genetic and physiological diversity of rhizobacteria from degraded land grown woody plants, and their effect on the establishment of key plant species in representative field plot with the application of OAs in North Shewa Zone, Oromia National Regional State, Ethiopia.

- **Specific Objectives**

The specific objectives of the current work are to:

- Assess the effect of organic amendments (OAs) application on soil physicochemical properties and determine the growth of alfalfa and grass pea under greenhouse conditions.
- Characterize the phenotypic and genetic diversities of rhizobacteria isolated from degraded soil through BOX-PCR and partial sequencing of 16S rRNA genes.
- Evaluate the effect of inoculating *A. abyssinica* seedlings with PGPR isolated from degraded sandy clay loam soil of Ethiopia, aiming at their use to enhance drought stress tolerance
- Determine phytobeneficial traits of PGPR and also to determine their effect in enhancing germination of *A. abyssinica* seeds.
- Measure microbial abundance and multipurpose tree establishment on degraded land before and after OAs and bacterial inoculations.

Chapter 2

2. Literature Review

2.1. The Problem of Land Degradation

Ecosystem services (ESS) are defined as the goods and benefits humans gain from healthy and functional ecosystems. Global change processes such as large-scale land-use change and climate change increase pressure on ecosystem functions and threaten the sustainable supply of ESS. Natural or human-induced factors that directly or indirectly cause a change in an ecosystem are potential drivers of changes in ecosystems (Reid *et al.*, 2005).

The Millennium Ecosystem Assessment (MA), a major UN-sponsored effort (2005) from 2001 to 2005, and identified four major categories of ecosystem services: provisioning, such as the production of food and water; regulating, like the control of climate and disease; supporting, like nutrient cycles and oxygen production; and cultural, such as spiritual and recreational benefits (Wallace, 2007).

Natural and man-made global land degradation is a reduction or loss in the biological and economic productive capacity of the land that undermines the delivery of ecosystem goods, and services (Higginbottom and Symeonakis, 2014). LD has become a global problem occurring in most terrestrial biomes and agroecologies, in both low-income and highly industrialized countries. Its effects on ecosystem services challenge conservation, management, and rehabilitation activities (Dubovyk, 2017).

The concept of LD stems from the loss of biological productivity of an ecosystem due to long-term loss of natural vegetation or depletion of soil nutrients (López-Ballesteros *et al.*, 2018). It harms global agriculture, food security, ecosystems, and severely impacts the livelihood of many people directly depending on agriculture around the world (Hazell and Wood, 2008). According to Nkonya *et al.,* (2016), about 30 % of the global land area is partly or completely degraded and 3 billion people are affected. Wang *et al.* (2016) indicated that, 24 to 75 billion tons of fertile soil from arable land has been lost due to soil erosion. The rate of top soil removal is more than 10 million ha per year (Le *et al.*, 2016).

Approximately 40% of land degradation has occurred in developing countries, which are projected to experience 78% of the global dryland (Huang *et al.*, 2016). Marble and Fritschel (2014) projected that if the current scenario of LD continues over the next 25 years, it may reduce global food production by 12% and a 30% increase in world food prices. There is growing evidence that biodiversity loss has a significant impact on the ecosystem and functions as climate change does or will (Brook *et al.*, 2013).

Currently, the cost of LD reaches about US$490 billion per year, much higher than the cost of action to prevent it (Nkonya *et al.*, 2013). The major causes of global biodiversity loss are anthropogenic and are exacerbated by ongoing climate change Warren *et al.* (2013), particularly because climate change accelerates habitat destruction, overexploitation, and the prevalence of invasive species (Brook *et al.,* 2008).

As biodiversity is lost and ecosystems are degraded, the bio-capacity of the planet to support living organisms reduces. As bio-capacity decreases, there are diminishing resources available to support a growing human population and increasing collective ecological footprint. As a result of soil degradation, it is estimated that about 11.9 -13.4% of the global agricultural supply has been lost in the past five decades (Eni *et al.*, 2010). Besides, soil degradation is also associated with off-site problems of sedimentation, climate change, watershed functions, and changes in natural habitats leading to loss of genetic stock and biodiversity (Jie *et al.*, 2002).

2.2. Situations of Land Degradation in Ethiopia

The principal environmental problem in the Eastern African highlands is LD, manifested mainly in the form of soil erosion, gully formation, soil fertility loss, water scarcity, and reductions in crop yield, which has been more pronounced in Ethiopia (Seifu Tilahun *et al.*, 2013). Although Ethiopia is endowed with enormous land resources it has been affected by multifaceted environmental problems including land degradation and declining biodiversity (Alebachew Gebrie, 2015). It has a high level of soil erosion among the SSA countries (Tesfa and Mekuriaw, 2014). Land degradation, especially in the highlands, has been identified as the most serious environmental problem in the country (Merkineh Mesene, 2017).

The Ethiopian highland studies revealed that 44% of the country's total highland area is seriously threatened by soil and biological degradation and has been identified as the most serious problem (Aune *et al.*, 2001). Some 27 million ha, representing approximately 50% of the highlands, are have been significantly degraded, and of this area, 14 million ha are badly eroded and if the present trend of soil degradation continues, per capita income in the highlands will fall by 30% in 20 years. (Genene Tsegaye, 2006) predicted that around 54% of the remaining highlands are highly susceptible to erosion.

In Ethiopia, land degradation, low and declining agricultural productivity, and poverty are severe and interrelated problems that appear to feed off each other (Nigussie Haregeweyn *et al.*, 2015). If urgent measures are not taken to arrest Ethiopia's serious land degradation, the country is headed for a "catastrophic situation" (Pender and Gebremedhin, 2006). Any effort towards this direction should begin from research that aims at exploring location-specific factors influencing the adoption of land management practices.

People in developing countries utilize land and soil resources in an unsustainable way as evidenced by overgrazing, destruction of forest for urban expansion, and highly intensive and unscientific agricultural activities, and the resulted in improper land-use/land-cover changes (De Meyer *et al.*, 2011). Hurni (1985) predicted that degradation and loss of soil resulting from soil erosion were estimated to be about 20 t per hectare in Ethiopia, i.e., about 1 mm of soil depth per year. Recent estimates by Hurni *et al.* (2015) indicated rates of 20 $\mathrm{Mg\,ha^{-1}\,year^{-1}}$ on currently cultivated lands and 33 $\mathrm{Mg\,ha^{-1}\,year^{-1}}$ on formerly cultivated degraded lands.

Ethiopia loses about 1.9 billion metric tons of fertile soil from the highlands every year and the degradation of land through soil erosion is increasing at a high rate (Pender *et al.*, 2012). The Ethiopian highlands reclamation study also forecasted that soil erosion would cost the country 1.9 billion USD between 1985 and 2010 (Berry, 2003).

Today, Ethiopia has entered a new period of restoration practices where large areas of degraded forestlands are put under rehabilitation, although deforestation has not been abated. Some land restoration practices, such as green manure (cover crops) has been lessening the degradation process (Nunes *et al.*, 2012). Forest rehabilitation in Ethiopia includes different types of strategies and actors. Various forms of agroforestry, reforestation/afforestation, area enclosure, and woodlot development are popular strategies of restoration observed today (Mulualem Tigabu and Demel Teketay, 2014).

2.2. Causes of Soil Degradation

The land degradation is caused by excessive pressure on land to meet the competing demands of the growing population for food, fodder and fiber (Aulakh and Sidhu, 2015). There are different causes for the different types and forms of land degradation. Soil quality decline is one of the main causes of land degradation and is considered to be responsible for 84% of the ever-diminishing acreage.

Year after year, huge acres of land lost due to soil erosion, contamination, and pollution (Mishra *et al.*, 2015). Soil degradation implies a decline in soil quality with an attendant reduction in ecosystem functions and services (Lal, 2009). Soil degradation may be caused by unfavorable alterations in one or all of a soil's physical, chemical and biological properties and processes (Figure 2.1) (Lal *et al.*, 1989).

There are several physical factors contributing to soil degradation distinguished by the manners in which they change the natural composition and structure of the soil. Soil physical degradation generally results in a reduction in structural attributes including pore geometry and continuity, thus aggravating a soil's susceptibility to crusting, compaction, reduced water infiltration, increased surface runoff, wind and water erosion, greater soil temperature fluctuations, and an increased propensity for desertification (Lal, 2015b). Physical processes of soil degradation led to changes in soil physical, mechanical, hydrological and rheological properties which have a negative effect on crop and animal production, farm income and environmental quality (Lal *et al.*, 1989).

Soil chemical degradation is characterized by acidification, salinization, nutrient depletion, reduced cation exchange capacity (CEC), increased Al or Mn toxicities, Ca or Mg deficiencies, leaching of NO3-N or other essential plant nutrients, or contamination by industrial wastes or by-products (Lal, 2015b). In the broadest sense, it comprises alterations in the soil's chemical property that determine nutrient availability. Chemical degradation processes include changes in soil's chemical properties that regulate nutrient activity and capacity; or which maintain a favorable balance among principal nutrient elements, and the accumulation of substances possibly to toxic concentrations. Soil chemical degradation leads to a reduction in a soil's ability to inactivate toxic compounds (Lal *et al.*, 1989).

Biological factors refer to the human and plant activities that tend to reduce the quality of the soil. Some bacteria and fungi overgrowth in an area can highly impact the microbial activity of the soil through biochemical reactions, which reduces crop yield and the suitability of soil productivity capacity (Aulakh and Sidhu, 2015; Lal, 2015b). Soil biological degradation reflects depletion of the soil organic carbon (SOC) pool, loss in soil biodiversity, a reduction in soil C sink capacity, and increased greenhouse gas (GHG) emissions from soil into the atmosphere. One of the most severe consequences of soil biological degradation is that soil becomes a net source of GHG emissions (*i.e.*, CO_2 and CH_4) rather than a sink (Lal, 2015b). Solomon Abate (1994) reported that soil organic matter content dropped from 20% to 7% in less than three years of continuous cultivation due to mineralization.

Ecological degradation reflects a combination of other three, and leads to disruption in ecosystem functions such as elemental cycling, water infiltration and purification, perturbations of the hydrological cycle, and a decline in net biome productivity (Lal, 2015b). The overall decline in soil quality, both by natural and anthropogenic factors, has strong positive feedbacks leading to a decline in ecosystem services and reduction in nature conservancy.

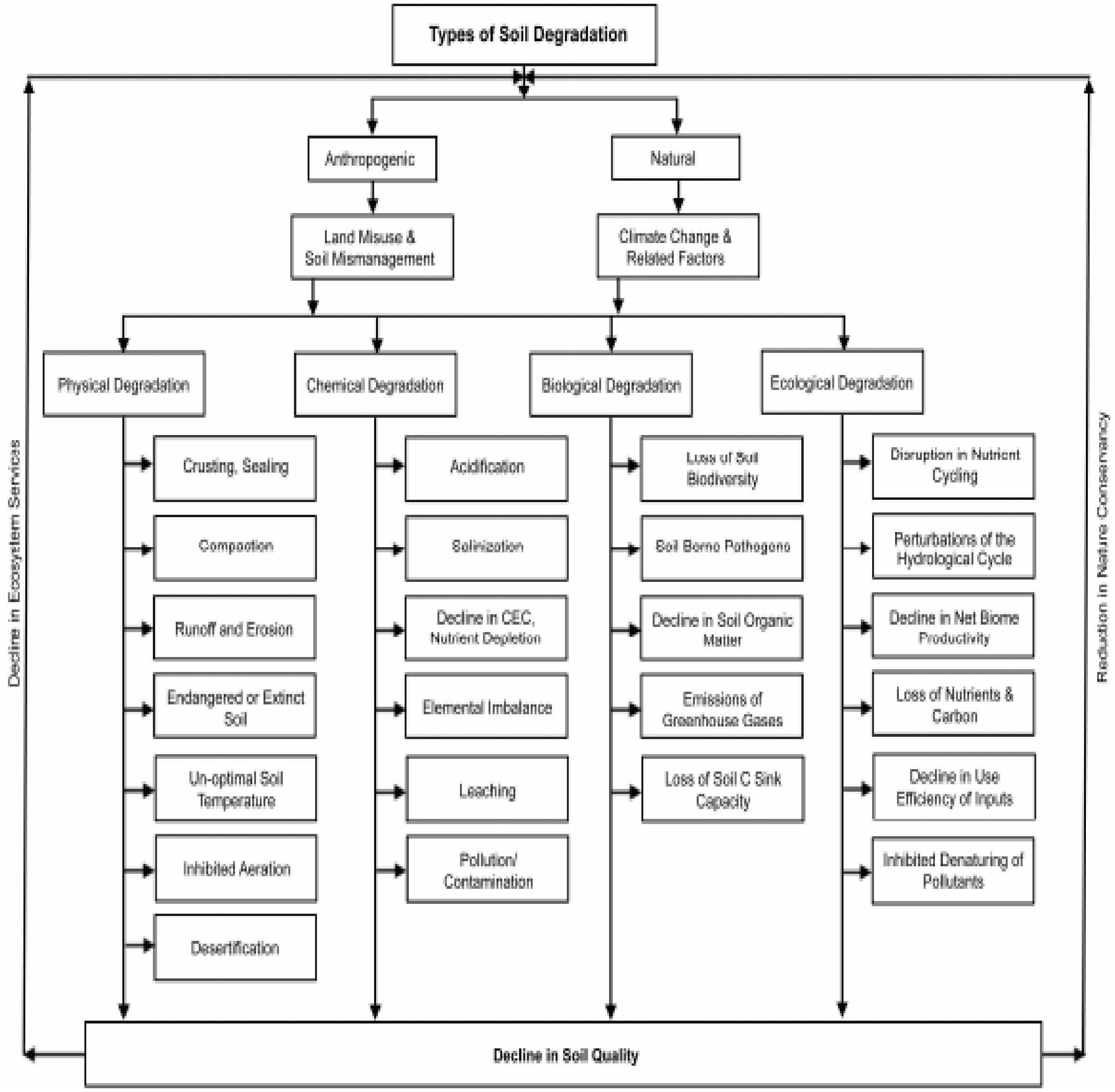

Figure 2.1. Causes and **t**ypes of soil degradation. Adapted from (Lal, 2015b)

2.3. The role of closures and plantations for restoration and rehabilitation of land degradation

Multi-purpose tree plantations with different species play an important role in restoring productivity, ecosystem stability, and biological diversity to degraded lands. Plantations using appropriate tree and shrub species are important for rehabilitation (Parrotta, 1992). Rehabilitation is the process of ecological re-establishment of vegetation and trophic structure in a destroyed ecosystem mainly due to anthropogenic activities. Likewise, restoration is defined as a tactic employed to return degraded lands to their original condition, and rehabilitation activities aim to repair ecosystem functioning with less emphasis on the recovery of structure and composition and more on increasing productivity for the benefit of people (Clewell and Aronson, 2013). Thus, rehabilitation efforts are more relevant and have multi-use with many proven approaches and technologies to progress from a less desired to a more desired ecosystem state.

Plants are the sole producers that support all the trophic levels and sustain life on earth and interact with both biotic and abiotic factors (Joshi, 2018). Plants exist in varied ecosystems and interact with varied organisms ranging from antagonistic organisms to beneficial organisms (Pieterse *et al.*, 2013). Consequently, these interactions play large and varied roles in the establishment and maintenance of plant community diversity and ecosystem properties (Larimer *et al.*, 2010).

Plant-soil interactions are the foundation of effective and sustained restoration of terrestrial communities and ecosystems. These interactions can vary greatly depending on their context (environmental conditions, management practices, time, neighboring community, interaction with other organisms) (Eviner and Hawkes, 2008).

2.4. Soil-Plant-Microbe (Tripartite) Interactions: Implications to Rehabilitation of Degrade land

Soil represents the major resource of the terrestrial ecosystem. In the natural environment, plants interact with the rich ecosystem that harbors a diverse group of microorganisms in the soil (Jacoby *et al.*, 2017). Terrestrial ecosystems across the globe are currently encountering declines in their biodiversity and ecosystem functioning due to land degradation and climate change (Theobald, 2010). Efforts to restore degraded lands primarily focus on the establishment of the aboveground plant communities on the soil.

The soil is one of the major reservoirs of biological diversity on the planet (Swift *et al.*, 1998). Many processes and interactions take place in the soil, contributing to a considerable number of ecosystem services (Coleman and Whitman, 2005). Thus, it is a habitat where plant roots and microorganisms form tripartite mutualistic and beneficial interactions with one another (Figure 2.2) (Glick *et al.*, 2007a). The tripartite interaction is observed in the natural environment. Both plants and microorganisms obtain their nutrients from soil and change soil properties by organic litter deposition and metabolic activities, respectively (Figure 2.2).

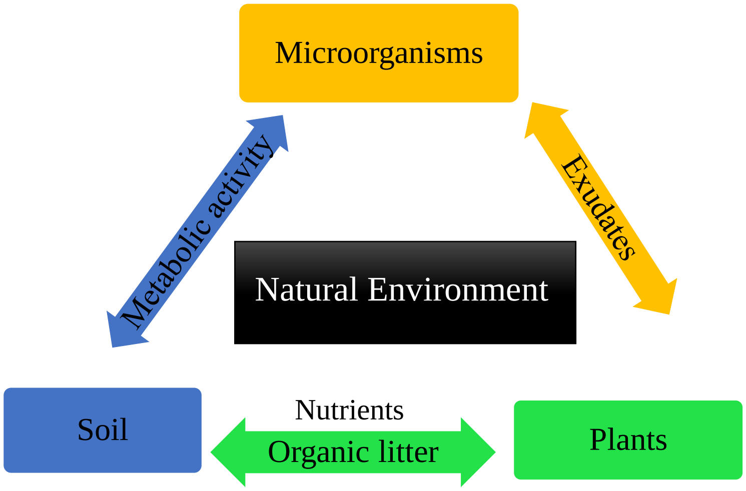

Figure 2.2. Interactions between plants, microbiota, and soil (Adapted and modified from (Jacoby et al., 2017)

The root area that is under the influence of plant roots is known as rhizosphere where very important and intensive interactions take place between the plant, soil, and microorganisms known as tripartite interaction (Figure 2.2) (Felestrino *et al.*, 2017). These microorganisms that enhance plant growth and health through various mechanisms are known as plant growth-promoting microorganisms (PGPM).

The plant microbiome stimulates germination and plant growth, enhances nutrient acquisition, and promotes direct and indirect mechanisms of defense against pathogens and abiotic stress, and influences plant phenology (Berg *et al.*, 2015). Many of these functions might be indirectly influenced by the microbial interactions that occur in the rhizosphere because the resulting plant microbiome could be functionally different (Barea *et al.*, 2011).

Plant-microbe relationships are pivotal for plant growth and survival (Van Der Heijden *et al.*, 2008). These interactions in the rhizosphere are the determinants of plant health, productivity, and soil fertility, and serves as a reservoir of additional genes that plants can access when needed (Rout and

Southworth, 2013). They also play vital roles in the maintenance of plant and soil productivity under biotic (diseases) and abiotic (salinity, drought, acidity, *etc.*) stresses (Vimal *et al.*, 2017).

In general, three mechanisms are usually put forward to explain how microbial activity can boost plant growth, health, and establishment in soil habitat: (1) manipulating the hormonal signaling of plants Verbon and Liberman (2016); (2) repelling or outcompeting pathogenic microbial strains Mendes *et al.* (2013); and (3) increasing the bioavailability of soil-borne nutrients (Van Der Heijden *et al.*, 2008).

Soil microbial community structure and composition measures are increasingly being used to assess ecosystem responses to anthropogenic disturbances and to provide an indicator of ecosystem recovery (Lewis *et al.*, 2010). They can respond more rapidly to changes in environmental conditions than plant communities and may provide an early indication of the recovery trajectory (Harris, 2009). However, the high level of sensitivity to numerous environmental factors can also result in long-term shifts (in the order of decades or more) in the microbial community structure in rehabilitated ecosystems (Jangid *et al.*, 2010).

The ever-increasing population and concomitant land degradation, global climate change, shrinking agricultural lands have collectively affected crop production and ecosystem services (Rashid *et al.*, 2016). The status of the microbial ecosystem can be assessed through the genetic characteristics of the soil microbial community and it also gives the idea about the quality of the soil and the progress of restoration after degradation (Singh, 2015).

The PGPR and mycorrhizae are key components of soil microbiota that play vital roles in the maintenance of plant fitness and soil health under stressed environments (Vimal *et al.*, 2017). The use of microorganisms in improving nutrient availability to plants is an important strategy and related to climate-smart practices (Hamilton *et al.*, 2016).

One of the most promising techniques to achieve these benefits is the use of bio-inoculants also called biofertilizers, which are composed of selected beneficial microorganisms. The application of beneficial microbes is already used in organic farming systems (Bender *et al.*, 2016). Therefore, the use of plant-associated microbes can be a useful approach for biotechnological application in plant production (Vargas *et al.*, 2019).

2.4.1. Diversity of important plant growth-promoting microorganisms in the rhizosphere

2.4.1.1 Rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are soil bacteria that colonize the surface and inner tissues of roots and promote plant growth and health (Dimkpa *et al.*, 2009). Alemayehu Getahun *et al.* (2020c) finds diverse rhizobacteria (*Pseudomonas*, *Bacillus*, *Serratia*, *Pantoea*, *Paenibacillus*, *Acinetobacter*, and others) from *A. abyssinica* and *J. procera* grown on degraded soil of North Shewa Zone, Ethiopia. Diverse genera of rhizobacteria are used for maintaining soil fertility include *Azospirillum*, *Bacillus*, *Klebsiella*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Enterococcus*, *Rhizobium sp*., and Mycorrhiza are to mention a few (Solanki *et al.*, 2017; Xing *et al.*, 2016). Mechanisms by which PGPR stimulates plant growth are broadly categorized as direct or indirect (Glick, 1995).

Degraded soils are often the targets of restoration efforts and are usually planted with native or exotic perennial plants, but without including specific microorganisms the restoration attempt would often fail (Bainbridge, 2012; Cowie *et al.*, 2011). Consequently, the application of microbial inoculants to the plant rhizosphere has been demonstrated to be a suitable strategy for revegetation purposes (Armada *et al.*, 2015; Mengual *et al.*, 2016). The rhizobial inoculants aggressively colonize the rhizosphere/plant roots and enhance the growth of plants when applied to the seed or drench inoculated to plants. Efforts to restore degraded lands with plants inoculated with microorganisms were carried out previously in different countries around the world.

Kim *et al.*, (2012) and de-Bashan *et al.*, (2012) showed that if native plants are inoculated with PGPR and/or mycorrhizal fungi, revegetation of degraded soils accelerates and soil health indicators improve. Other studies also indicated that AMF inoculation enables plants to cope with drought stress by overcoming the detrimental effect of water and nutrient limitations (Bashan *et al.*, 2009). It is also established that ecosystem functioning is largely governed by the microbial activity of both soil bacteria and AMF, particularly autochthonous strains that are adapted to specific environmental conditions (Azcón *et al.*, 2010; Kim *et al.*, 2012).

The re-establishment of shrubs and tree species is an essential strategy for recovering degraded soils. Thus, in the process of land rehabilitation, PGPR deserves special attention as it is actively involved in plant and soil interactions. In semiarid areas of southern Spain, a combination of arbuscular mycorrhizal (AM) fungi, rhizobia, PGPR, and yeasts was used to promote the growth of plants and enhance soil fertility (Armada *et al.*, 2014; Pérez-Fernández *et al.*, 2016)

A field assay was carried out to evaluate the effect of multiple inoculations of native rhizobacteria (*B. megaterium, Enterobacter* sp., *B. thuringiensis,* and *Bacillus* sp.) for the establishment of *Lavandula dentata L*. with a remarkable synergetic effect on the host plant (Mengual *et al.*, 2014). *Bacillus* is the most abundantly represented genus in the rhizosphere and many strains have been known as important plant growth-promoting bacteria for many years by increasing nutrient availability of the plants (Charest *et al.*, 2005). *B. subtilis* naturally present near plant roots, can maintain stable contact with higher plants and promote their growth. Moreover, del Carmen Jaizme-Vega *et al.* (2004) also suggested that *Bacillus* sp. can be described as a future way to increase plant health and survival rates in commercial nurseries.

Pseudomonas spp. is invariably present in agricultural soils having traits that make them well suited as PGPR, and are functionally and metabolically most diverse and help in the maintenance of soil vigor. The most effective strains of *Pseudomonas* have been *Pseudomonas fluorescens*. *Pseudomonas* help in the maintenance of soil vigor and are functionally and metabolically most diverse (Lugtenberg and Kamilova, 2009). Johri (2001) had demonstrated on field trials that *P. putida* GRP3 led to a massive increase in the yield of legumes.

Other studies also showed the co-inoculation of *Azospirillum brasilense* and *Bacillus pumilus* significantly promoted the growth and development of *Atriplex lentiformis* growing on nutritionally poor mine tailings in the southwestern USA (De-Bashan *et al.*, 2010a; De-Bashan *et al.*, 2010b). Another study showed that several different species of PGPR promoted the ectomycorrhizal symbiosis of *Acacia holosericea* in southern Senegal (Duponnois and Plenchette, 2003).

Likewise, native trees inoculated with a consortium of PGPR performed well in degraded parkland soils of India Ramachandran and Radhapriya (2016), and rock weathering bacteria supported plant growth in China (Wu *et al.*, 2017b). Furthermore, in southern Mexico and Argentina, inoculation with the species *A. brasilense* significantly promoted the growth, establishment, and survival of several legume trees and cacti in desert soils (Bashan *et al.*, 2009; Bashan *et al.*, 2012). All taken together, these studies indicated that to promote plant growth and health for the rehabilitation of degraded lands, it is essential to undertake extensive research efforts on microbial diversity, their distribution, as well as function in soils of degraded lands.

2.4.2. The Role of Organic Amendments (OA) for Land Rehabilitation

Degraded habitat is characterized by the removal of organic matter and the nutrient-rich layer of soil profile causes nutrient depletion, loss of soil fertility, structure, and water holding capacity

(Montgomery, 2007). One of the options to halt these problems is the application of OAs that include diverse techniques ranging from mulch to organic waste; compost, animal manures, crop residue, and biochar and bio-fertilizers (Larney and Angers, 2012; Luna *et al.*, 2016). A soil amendment is any material added to soil to improve its physical, chemical, and biological properties. The goal is to provide a better environment for plant roots (Davis and Whiting, 2013).

The common characteristic of soil OAs is that they contain organic matter to a certain concentration, and are important in soil nutrient management including the macro-and micronutrient status of the soils (Barnawal *et al.*, 2014). The combinations of different types of amendments have demonstrated better results than their single application (Alemayehu Getahun *et al.*, 2020b; de Varennes *et al.*, 2010). The addition of animal manure, green manure, compost, and biochar have been instituted to arrest the degradation process.

These practices have increased the amount of soil cover and improved the physical properties of the soil (Nunes *et al.*, 2012). Cerdà *et al.* (2016) assessed the use of OAs as a restoration technique for better vegetation establishment and increased soil fertility to enhance vegetation growth and development. Amending soil with organic materials is a promising strategy to build-up C levels in the paddy soils of subtropical China (Xie *et al.*, 2016). Page-Dumroese *et al.*, (2018) proposed that biochar, manure, compost, and bacterial inoculations are an effective method to improve the physical properties of soils and accelerate re-vegetation and rehabilitation.

2.4.2.1. Biochar for land Rehabilitation

Biochar is a carbon-rich biological material combusted under low oxygen conditions with remarkable benefits as a soil amendment (Lehmann and Joseph, 2015). It positively impacts an array of soil processes ranging from benefiting soil biology, controlling soil-borne pathogens, enhancing nitrogen fixation, improving soil physical and chemical properties, decreasing nitrate (NO^-_3) leaching and nitrous oxide (N_2O) emission (Najar *et al.*, 2015).

It can also affect key physical and chemical parameters of soil, (soil pH, structure, the release of soluble C, and micronutrient availability), which in turn influence microbial community structure and functions (Chintala *et al.*, 2014). Its application increased agricultural yield Major *et al.* (2010), reduce pollution Fellet *et al.* (2011), sequester Carbon in the soil Galinato *et al.* (2011), and restore organic matter content in degraded soils or rocky substrates (Anawar *et al.*, 2015).

Biochar can alter the surface area, pore space, bulk density, water-holding capacity, and penetration resistance of the soil Mukherjee *et al.* (2014), improve soil moisture availability (Sohi *et al.*, 2010). It is a very important input for the rehabilitation of degraded land, converting poor soils for agricultural production (Barrow, 2012). Although biochar cannot be used as a fertilizer, it can be applied to the field every year and serve as a slow-releasing reservoir of nutrients in soils (Hossain *et al.*, 2011).

In general, there is a lot of interest in the potential of biochar for enhancing C sequestration and improving soil fertility Spokas *et al.* (2012), restoring degraded lands Beesley *et al.* (2011), and reducing greenhouse gas (GHG) emissions in agriculture (Cayuela *et al.*, 2014). These benefits provide the basis for recent calls for broader, even global upscaling of biochar use for environmental protection and agricultural production (Cernansky, 2015).

2.4.2.2. Compost as an organic amendment for Rehabilitation

Compost is the most common organic soil amendment. Its application to soil is used to maintain and improve soil structure Lillenberg *et al.* (2010), and reduces N_2O emissions compared to mineral fertilizers because most N is in organic form is converted to NO_3 (Alluvione *et al.*, 2010). Compost amendments to soil increased organic matter, and N, P, and K content of the soil implying improved biomass production and nutrient uptake before the establishment of vegetation, soil restoration, and regeneration (Benedek *et al.*, 2012; Miller *et al.*, 2015).

Generally, nutrient deficiencies and poor soil fertility have emerged as major constraints in the sustainable production in agriculture on a global scale. The use of biochar and biochar-compost mixtures from different organic sources is all the more important for improving soil fertility, restoring degraded land, and mitigating the emissions of greenhouse gasses associated with agriculture (Getachew Agegnehu *et al.*, 2017).

2.4.2.3. Manure for the rehabilitation of degraded land

Manure is the cheapest and easily accessible nutrient source to improve resources and is regarded as soil fertility (Gavili *et al.*, 2018). The main sources of manures are pig, duck, sheep, chicken, and cattle farms. Khan *et al.* (2017), estimated that about 7 x 10^9 Mg (mega gram) animal manure is generated all over the world every year. It is an organic amendment that provides nutrients for crop production, reloads soil organic matter lost through erosion, and influences chemical, physical, and biological properties of soil (Larney and Angers, 2012).

Other reports showed manure applications display a decrease in soil bulk density, increase soil water retention, and improve soil hydraulic properties, soil pH, SOC, N, and P availability (Mikha *et al.*, 2014). Farmers preferentially apply manure to fields closest to homesteads (home fields), which are more fertile than fields further away (outfields) (Zingore *et al.*, 2008). Acosta-Martinez *et al.* (2011) emphasized the great potential benefits of manure application on eroded land that enhances soil biogeochemical cycling necessary for agroecosystem production.

Another report showed that amending degraded soils with organic manure improves soil quality, especially in urban areas where trees are grown for the rehabilitation of damaged soils (Wiseman *et al.*, 2012). All taken together, organic amendments with compost, biochar, and others are receiving attention in the context of restoring disturbed ecosystems soils to improve tree growth and address environmental degradation.

2.4.3. Cover crops for rehabilitation

Cover crop improves soil quality by improving soil organic matter content. The term “green manure/cover crop” is coined to describe planting different species of plants, with different habits; tree, a bush, a vine, a crawling plant or algae, to maintain or improve soil fertility or control weeds (Altieri and Toledo, 2011). Continuous vegetative cover such as residue mulch and cover cropping is one of the site-specific techniques of restoring soil quality (Lal, 2015a).

Green manures/cover crops are capable of adding as much as 50 metric tons/hectare (MT/ha) or more of organic matter (green weight) to the soil each year (Chasek *et al.*, 2015). This organic matter has various positive effects on the soil, such as recycling nutrients back into the soil, pumping nutrients up to the soil surface, and improving the soil’s water-holding capacity (Bunch and Kadar, 2004). Cover crops are usually leguminous crops grown to improve soil health by guaranteeing permanent soil cover by fixing atmospheric nitrogen and adding organic matter to the soil.

Green manures and cover crops serve as mulch to the soil to prevent the soil from wind/water erosion and moisture loss. Different studies indicated that establishment and management of forage trees (i.e., *Acacia fadherbia*) Garrity *et al.* (2010) and grass-legume mixtures, Muir *et al.* (2011) improve health and/or quality of rangeland and agricultural soils, and reduce or reverse land degradation by increasing biomass content and biodiversity (Veum *et al.*, 2015).

One study showed that farmers cultivating maize and a legume, *Mucuna* increased the productivity of the soil on hillsides of northern Honduras, with a 35% slope and more than 2,000 millimeters of annual rainfall year by year, without using any other soil conservation practices (Buckles *et al.*, 1998). This is because the soil is protected from erosion for it is covered by *Mucuna* 10 months of the year.

Alfalfa (*Medicago sativa* L.) is one of the world's most versatile crops. Growers admire alfalfa for its high yield, wide adaptation, disease resistance, and excellent feeding quality. Alfalfa makes a tremendous contribution to world food production, a contribution that often goes unrecognized (Shi *et al.*, 2017). Alfalfa plays an important role in erosion control, weed suppression, drying wet soils, nitrogen management and improving soil quality (Dinnes *et al.*, 2002)

Grass pea (*Lathyrus sativus* L.) is widely cultivated for food and feed in some developing countries including Ethiopia (Mohammed *et al.*, 2020). Grass pea is one of the pulses suitable for relay cropping. It has potential among grain legumes for its tolerance to harsh conditions and its adaptability to unfavorable environments with little disease or insect problems. Grass pea has been grown as a cover crop, generally cultivating towards the end of the monsoon rice harvest. This system exploits the ability of grass pea to grow in swampy conditions but also, its drought tolerance, utilizing residual moisture in the paddy fields and growing to maturity during the dry season (Das, 2000; Nazrul and Shaheb, 2015). Grass pea besides the nutritional benefits, has an important role as a legume crop in crop rotations, reportedly adding around 67 kgha-1of nitrogen to the soil from symbiosis with *Rhizobium sp.* in a single season and conferring yield and protein benefits on the subsequent non-legume crop (Jennifer, 2003). Generally, on top of this, the application of OAs showed a better soil fertility increment and cover plants (alfalfa and grass pea) growth parameters under field and greenhouse experiments (Alemayehu Getahun *et al.*, 2020b).

2.5. Ecological diversities of PGPR

The growth performance of the bacterial strain's community depends on the growth properties. Their phenotypic profiling such as the ability to utilize different C and N sources revealed the metabolic versatility of the selected strains of the bacteria (Li *et al.*, 2017). Carbon source utilization can determine the ability of an organism to live symbiotically or saprophytically in the soil (Brückner and Titgemeyer, 2002). The metabolic capabilities of strains depend on their growth rates and ability to metabolize specific metabolites (Tzamali *et al.*, 2011).

The metabolic assets of an organism could contribute toward a particular adaptation and therefore might provide valuable information about bacteria supportive for root colonization (Mazur *et al.*, 2013). It is suggested that ecophysiological versatile strains are more successful competitors in extreme environments (Wielbo *et al.*, 2007). Phenotypic profiling is important for understanding genotype differences, stress responses, media composition, and changes in environmental conditions for microorganisms (Chojniak *et al.*, 2015).

2.6. Genotypic diversities of PGPR

The molecular techniques are often applied, these days, to investigate the genetic diversity of PGPR from degraded soil. Polymerase Chain Reaction (PCR)-based genotyping methods are suitable tools to examine microbial diversity (Jin *et al.*, 2011). The development of molecular biological techniques such as DNA sequencing, PCR fingerprinting, housekeeping genes, and BOX-PCR analysis have dramatically changed bacterial taxonomy and phylogeny (Oren and Garrity, 2014). For phylogenetic analysis, genes used as molecular taxonomic markers should be universal and not show any mutation to specific conditions Patwardhan *et al.* (2014), and in this regard, housekeeping genes are most suitable and useful for strain discrimination and identification of bacteria.

The evaluation of genetic diversity amongst soil bacteria is also indicated (Hayat *et al.*, 2013; Upadhyay *et al.*, 2009). The molecular RAPD-PCR technique was used to analyze the genetic variability among P-solubilizing PGPR. Phylogenetic analysis based on 16S rRNA analysis provided good discrimination for the better characterization of phosphate solubilizing bacterial isolates at the species level (Mehta *et al.*, 2015). BOX-PCR resulted in complex amplified banding patterns, reflecting a high degree of genotypic diversity among the beneficial bacteria. Likewise, the understanding of the molecular diversity of PGPR can be helpful in the selection of the dominant types of the bacteria involves in phytobeneficial role which finds their use as biofertilizers (Solanki *et al.*, 2017). Matos *et al.* (2005) indicated that microbial communities with greater microbial diversity would be less susceptible to invasion by pathogens and environmental stresses. Hence, a complete evaluation of genetic diversity and environmental influence can be useful for the introduction of new microorganisms into the environment to enhance plant rehabilitation in degraded land rehabilitation.

2.7. The role of PGPR in other Plant Stress Tolerance

Abiotic stresses are considered to be the main sources of agricultural yield reduction. However, the intensity of abiotic stress varies depending on the type of soils and plant factors (Nadeem *et al.*, 2010). Due to their sessile nature, plants have always been confronted with various abiotic and biotic stresses in their immediate environment. All plants are known to perceive and respond to stress signals such as drought, soil acidity, heat, salinity, herbivory, and pathogens (Hirt, 2009). PGPR can improve the growth of crops under abiotic stress conditions and might therefore open new applications for a sustainable agriculture (Egamberdieva and Kucharova, 2009). Bacteria belonging to the genera of *Rhizobium, Bacillus, Pseudomonas, Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum* and many others have been reported to provide tolerance to host plants under different abiotic stress environments (Grover *et al.*, 2011).

Drought stress is a key factor limiting crop production in the world and an increasing problem under current climate change. There is renewed interest in rhizosphere microorganisms capable of ameliorating plant stress via a wide range of mechanisms that span modification of plant hormone levels and production on bacterial exopolysaccharides (Kaushal and Wani, 2016; Naveed *et al.*, 2014). Drought is one of the major limitations to food production worldwide and is estimated to have reduced national cereal production by 9-10% (Lesk *et al.*, 2016). Plants have developed several mechanisms to cope with drought stress including morphological adaptations, osmotic adjustment, optimization of water resources, antioxidant systems that diminish the harmful effects of reactive oxygen species (ROS) linked to drought, and induction of a variety of stress-responsive genes and proteins (Farooq *et al.*, 2009). To date, creation of drought-tolerant cultivars has been the approach used to mitigate the negative effects of drought stress on crops and crop yields (Eisenstein, 2013). The approaches mentioned above overlook the ecological context of the soil environment in which the crops are grown. Therefore, there is a need for microbial based approaches to mitigate drought stress. Plant-associated microbial communities currently have received increased attention for enhancing crop productivity and providing stress resistance (Glick *et al.*, 2007b; Yang *et al.*, 2009).

The effect of PGPR in general and rhizobia specifically on plants under abiotic stress conditions is the improvement of leaf water status, especially under salinity and drought stress (Ahmad *et al.*, 2013). Sarma and Saikia (2014) reported that *Pseudomonas aeruginosa* strain has improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. Ahmad *et al.* (2013) and Naveed *et al.* (2014) reported that the stomatal conductance (water vapor exiting through the stomata leaf) of plant leaf was higher in PGPR inoculated plants than non-PGPR inoculated ones under drought conditions. The finding from both studies proves that PGPR-inoculated plants tend to improve the water-use efficiency of plants. This finding could be beneficial to the environment in terms of reducing excessive usage of water (Mnasri *et al.*, 2007). These data clearly illustrate the relationship between the osmotic stress tolerance of the bacterial strain and the efficiency of the symbiosis. Similar results were reported by Swaine *et al.* (2007), since they found that a strain of *Bradyrhizobium elkanii* isolated from a drought environment was more tolerant to an in vitro osmotic stress than strains isolated from wet environments. *Achromobacter piechaudii* ARV8 which produced ACC deaminase, conferred IST against drought and salt in pepper and tomato (Mayak *et al.*, 2004). Grover *et al.* (2014) reported that sorghum plants treated with PGPR, *Bacillus spp* strain KB 129 under drought stress showed 24% increase in RWC over plants that were not treated with PGPR. Similar results have been demonstrated in maize (Naveed *et al.*, 2014).

Salt stressed soils are known to suppress the growth of plants (Paul, 2013). In fact, almost 40% of the world's land surface is affected by salinity-related problems (Zahran, 1999). Enhanced salt tolerance of *Zea mays* upon co-inoculation with *Rhizobium* and *Pseudomonas* is correlated with decreased electrolyte leakage and maintenance of leaf water contents (Bano and Fatima, 2009). Marulanda *et al.* (2010) reported that *Bacillus megatertum* strain inoculated into maize roots increased the ability of the root to absorb water under the salinity conditions. Gond *et al.* (2015) also found similar behavior when *Pantoea agglomerans* was inoculated into the maize roots. They found that the ability of the maize root to absorb water in saline conditions has improved. Here, bacteria that can grow under hypersaline conditions will be better able to colonize the root rhizospheres and external spaces of roots that are themselves exposed to high salinity conditions. Thus, the strategy was to first screen the bacterial isolates for their ability to grow under hypersaline conditions. Gonzalez *et al.* (2015) used *A. brasilense* to improve the salt tolerance of the jojoba plant during *in vitro* rooting. Based on the findings obtained, *A. brasilense* can reduce the undesirable effects of saline conditions on the jojoba rooting. The bacteria attenuated salinity's effect on the rooting ability of the jojoba plant. This indicates that *A. brasilense* has higher plant tolerance to salt stress. Increased production of proline along with decreased electrolyte leakage, maintenance of relative water content of leaves and selective uptake of K ions resulted in salt tolerance in *Zea mays* co-inoculated with *Rhizobium* and *Pseudomonas* (Bano and Fatima, 2009). Fasciglione *et al.* (2015) found that inoculation with *Azospirillum* sp. not only improves lettuce quality but also extends the storage life of a lettuce grown under salt stress, which further improves the yield. Yao *et al.* (2010) also reported that inoculation with *P. putida* Rs198 promoted cotton growth and germination under conditions of salt stress.

Chapter 3

3. Effects of Organic Amendments on Soil Physicochemical Properties, Growth and Biomass Yields of Alfalfa and Grass pea Grown on Soil of Girar Jarso District

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Abstract

Land degradation is an endless challenge in the world including Ethiopia and severely threatens ecosystem services due to the progressive depletion of soil nutrients. Consequently, rehabilitation of highly degraded land using organic amendment is enormously vital and an urgent priority issue for the benefit of all life on Earth. The purpose of this study was to assess the effect of organic amendments (OAs) application on soil physicochemical properties and determine the growth of alfalfa and grass pea under greenhouse conditions. Biochar from the coffee husk, compost (recycled from floriculture), and manure (farmyard manure) was used as OAs. Soil samples were collected from nine random corners at depth of 30 cm to form a composite sample. The soil samples had sandy clay loam texture and were deficient in nutrients and organic matter content with acidic pH values. In each experiment, five different treatments were considered (biochar, compost, manure, mixed, and control) at a 1:1 ratio of OAs and soil per pot, arranged in a completely randomized design in triplicate. Similarly, the field experiment was made on a completely randomized block design and each block contained five 41 x 4 m plots assigned at random within the block and separated by 1 m walkways. The use of OAs increased soil pH (5.69-8.13), cation exchange capacity (43.78-49.98 $cmol_c/kg$), organic carbon (1.41-2.46%) and organic matter (2.43-3.91%), total nitrogen (0.13-0.76%), available P (18.9-26.31 ppm) and (iron, Fe, manganese, Mn, copper, Cu and zinc, Zn) in comparison to the non-treated soil. Likewise, combined treatment had the largest effect on the biomass of cover crops with 3.43 g fivefold of the control (0.7 g) in alfalfa and 4.54 g twofold of the control (2.07 g) in alfalfa and grass pea, respectively $p \leq 0.05$. Both in the field and greenhouse experiment combined application biochar and other organic amendments showed a better soil fertility increment and plant growth parameters. The study concluded that there is a synergistic effect in OAs on the soil fertility restoration and plant growth performance indicating the significance of organic residues be used as a practical alternative option to enhance the rehabilitation of degraded habitats.

Keywords: Cover crops, Coffee husk, Land degradation, Rehabilitation

3.1. Introduction

Soil fertility is one of the vital ecological elements for the survival of biota and environmental services which is described by the presence or absence of nutrients that have roles in the conservation of biodiversity and habitats (Lone *et al.*, 2016). Global land assets are severely threatened due to degradation and unsustainable land-use practices that need urgent calls to curb the continually increasing land deterioration in resources poor developing countries.

Rehabilitating degraded land is highly essential for regaining ecosystem services such as biodiversity renovation that ensures the perpetuation of future generations (Tripathi *et al.*, 2017). The aggravation of LD and restoration to its original state is one of the pronounced and growing concerns in the world (Tejada *et al.*, 2010). It takes place in all parts of the terrestrial world and leads to an alteration of ecological and monetary features due to a reduction in productivity and quality of the land (Naseer and Pandey, 2018).

The management of LD has been challenged by an increase in population and global climate change. Degraded lands are the center of attention as the world's demands for food, feed, and fuel are increasing whilst the agricultural land base needed for production is shrinking (Lambin and Meyfroidt, 2011). It has been estimated that LD is severely affecting ~1.5 billion humans and ~12.2 billion hectares of total land the world over (Abhilash *et al.*, 2016). Soil fertility degradation is certainly regarded as the main food security constraint in Sub-Sahara African countries in general, and Ethiopia, in particular (Verchot *et al.*, 2007).

Soil erosion, low agricultural productivity, and poverty are too critical and interrelated problems in the Ethiopian highlands (Yitbarek Tibebe *et al.*, 2012). Highland places are characterized by a high population, high rainfall, and by sloppy and fragile ecology. It is estimated that ~1 billion tons of topsoil is lost annually in Ethiopia due to soil erosion (Kirui and Mirzabaev, 2015). In Ethiopia, the annual costs of land degradation associated with soil erosion and nutrients loss from agricultural and grazing lands are estimated at $106 million (about 3% of agricultural GDP) from soil and nutrient losses (Kirui and Mirzabaev, 2016). All these translate to an annual total loss of about $139 million (about 4% of GDP).

Land restoration efforts have been underway in Ethiopia since the 1970s, and nowadays the country is dedicated to restoring 15 million hectares of degraded land in 2030 (Pistorius *et al.*, 2017). Degraded soils regularly contain low organic matter, nutrients, and microbial activity that led to poor plant establishment (Wong, 2003). Maintenance and enhancement of the quality of degraded lands are, in essence, dependent upon the improvement of physical, chemical, and biological properties (Barrow, 2012).

The application of organic materials to degraded soils improves deficiencies in nutrients and organic matter, soil porosity, and increases microbial biomass to enhance plant establishment (Sohi *et al.*, 2010). The addition of plant residues, manure, biochar, or compost to soil can have a positive effect on soil microbial communities and on plant nutrient supply for the proper function of the ecosystems that ultimately contribute to the restoration of soil fertility of a given habitat (Suprapta, 2012). There is increasing interest in amending degraded soils with biochar and other organic matter to enhance the provision of ecosystem services. They are receiving renewed attention in restoring disturbed soils and improve plant growth (Cogger, 2005). It is hypothesized that biochar and other amendments on degraded habitat are an inexpensive solution to increase soil function and accelerate re-vegetation and improve soil physicochemical characteristics. Hence**,** the major purpose of this study was to evaluate the effect of different OAs on the physicochemical properties of soil samples collected from degraded habitats and to assess also the growth of alfalfa (*Medicago sativa*) and grass pea (*Lathyrus sativus*) on organically amended soils under greenhouse conditions.

3.2. Materials and Methods

3.2.1. Description of the study area

The study was carried out on degraded soils of Central Highlands of North Shewa Zone, Girar Jarso Woreda, Torbenashe Kebele from September 2016 to May 2018. The experiments were carried out on soil with a sandy clay loam texture. Global Positioning System (GPS) was used to identify the geographical locations of the study site (Figure 3.1). The major soils of the North Shewa zone are Vertisols, Cambisols, Histosols, and Lithosols.

More than 65% of the area is characterized by steep-slopes and a mountainous topography with hills, valleys, and gorges. Its altitude extends from about 1000 located in Abay gorge in Wara Jarso district to over 3500 m.a.s.l. located in the Degem district (Meskerem Abi and Degefa Tolossa, 2015). According to Fiche Station meteorological data, the average rainfall of the Woreda is about 883 mm, and maximum and minimum rainfall is about 1115mm and 651 mm, respectively. The temperature of the Woreda ranges from a minimum of 11.5°C to a maximum of 35°C with the average relative humidity during the year is 62%.

Data from Woreda agriculture center indicated that cultivated land covers the largest share, 71 percent while grazing land is the second-largest land use pattern that covers 11 percent. About 8 percent of the Woreda's' land area is unusable (abandoned) land which is neither cultivated nor grazed this includes, rugged topography, mountainous, exhausted degraded land areas and 0.65% is another form of land (Meskerem Abi and Degefa Tolossa, 2015). In the study area, heavy rain started in June and ends in September and the dry seasons occurred from (October to January) which is followed by small rain (February to May).

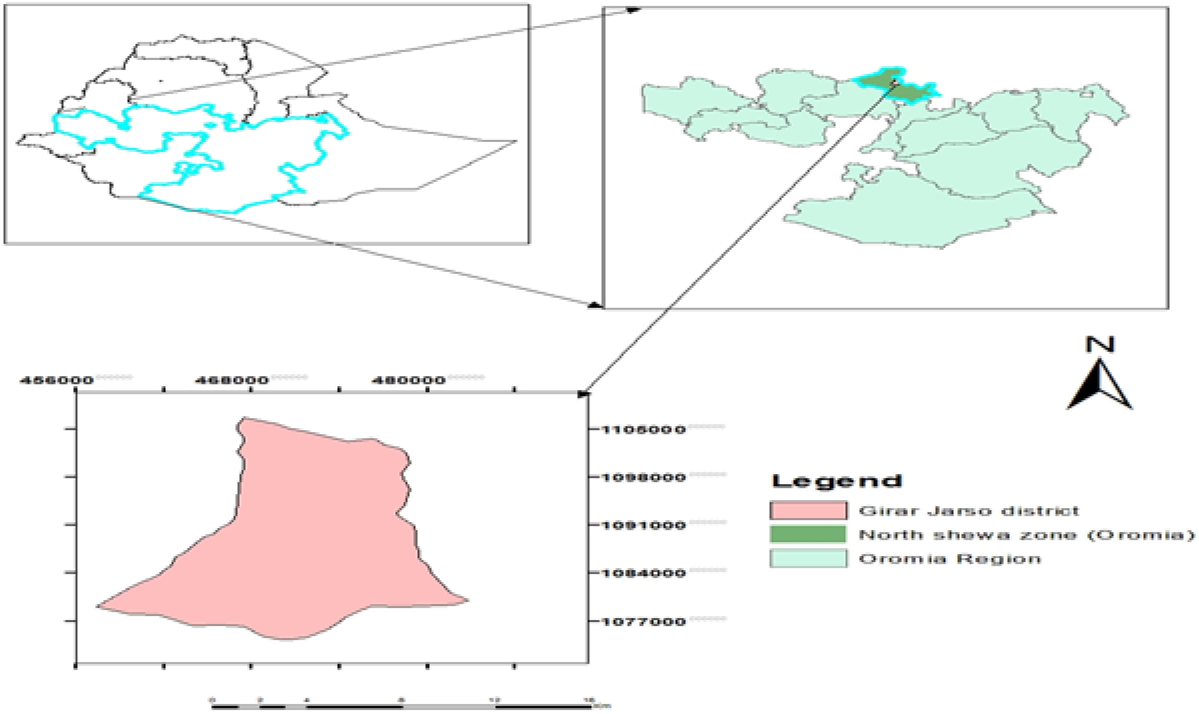

Figure 3.1. Map of study area

3.2.2. Soil collection

Four kilograms of soil samples were collected randomly from nine corners using zipped plastic bags from a depth of up to 30 cm and stored at room temperature. Thirty soil samples were collected. The soil samples were blended completely following a standard procedure (Margesin and Schinner, 2005). The selected properties of the soil samples are listed in (Table 3.1 and 3.2).

Table 3.1. Physicochemical properties of the soil before organic amendments

Property	Unit	Mean Values	Property	Unit	Mean Values
Moisture	%	9.2	CEC	cmolc/Kg	43.78
Depth	cm	0 -30	Ex. Na	cmolc/Kg	0.18
Altitude	M	3114	Ex. K	cmolc/Kg	0.18
Texture			Ex. Ca	cmolc/Kg	25.26
Clay content	%	31	Ex. Mg	cmolc/Kg	12.63
Silt content	%	21	Av. K	meq/100 g	0.14
Sand content	%	49	TN	%	0.13
Soil class	-	Sandy clay loam	OM	%	2.43
pH (H_2O)	pH meter	5.69	C: N	-	10.92
pH ($CaCl_2$)	pH meter	5.14	Cu	mg/Kg	2.52
EC	dS/m	0.25	Fe	mg/Kg	38.62
Av. P	Ppm	18.9	Zn	mg/Kg	0.59
OC	%	1.405	Mn	mg/Kg	4.08

Table 3.2. Average physical and chemical properties of applied biochar, compost, and manure (means ± standard deviation, n = 2).

Parameters	Biochar	Compost	Manure	P-values
Moisture	2.19 ^{b} (0.16)	3.13^{c} (0.12)	5.13$^{a\,(}$0.18)	0.001
pH (H_2O)	8.66^{b} (0.41)	8.45^{a} (0.41)	8.47$^{b\,(}$0.35)	0.85
EC	4.95^{b} (0.21)	1.95^{a} (0.58)	4.94$^{a\,(}$0.43)	0.01
Na %	1.74^{b} (0.09)	1.81^{b} (0.09)	1.985^{a} (0.05)	0.007
K %	0.05^{a} (0.014)	0.09^{a} (0.007)	0.12$^{a\,(}$0.12)	0.064
Ca %	3.39^{a} (0.19)	1.56^{a} (0.16)	2.36$^{a\,(}$0.08)	0.003
Mg %	1.35^{c} (0.0.09)	0.79^{b} (0.09)	2.24^{a} (0.10)	0.0014
Av. K (cmol$_c$/kg)	30.7^{c} (1.13)	25.6^{b} (1.74)	37.6$^{a\,(}$1.56)	0.01

TN %	1.2^{a} (0.18)	1.63^{b} (0.19)	$1.15^{b(}$0.05)	0.09
TOC	47.98^{a} (3.88)	22.57^{c} (1.22)	30.97^{b} (1.99)	0.035
Av. P (mg/kg)	1425.4^{c} (17.02)	$3238.37^{b(}$28.02)	5625.12^{a} (14.78)	0.00
Cu mg/kg	11.35^{b} (0.54)	16.65^{c} (0.44)	30.11^{a} (1.04)	0.0003
Fe mg/kg	2102.6^{a} (56.9)	1998.77^{a} (54.87)	1290.05^{b} (2.19)	0.007
Zn mg/kg	102.61^{c} (0.75)	82.99^{b} (0.61)	161.1^{a} (0.34)	0.00
Mn mg/kg	138.26^{a} (1.94)	$1.31.39^{a}$ (1.36)	709.95^{a} (01.17)	0.00

EC= electrical conductivity, TN = total nitrogen, TOC= total organic carbon, Av = available. Means with the same letter across the rows are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD)

3.2.3. Sources of organic amendments

Biochar (coffee husk), compost (recycled from floriculture) was obtained from Soil & More Ethiopia (SME), and manure (farmyard manure) was gathered from the farm.

3.2.4. Alfalfa and grass pea seeds

The seeds of alfalfa (*Medicago sativa*) Peruvien- DZF- 406 cultivar and grass pea (*Lathyrus sativus*) of wasse variety were obtained from the Ethiopian Biodiversity Institute (EBI).

3.2.5. Soil and OAs analysis

The composite soil samples and OAs were air-dried, ground, and sieved through 2 mm mesh and used for soil physicochemical analysis. Soil pH and electrical conductivity (EC) were measured in soil: water suspension (1:2.5) ratio (Joshi *et al.*, 2009). Cation exchange capacity (CEC) was determined by Sodium equivalent by Flame Emission Spectrophotometer (FES) (Sahlemedhin Sertsu and Taye Bekele, 2000). Ammonium acetate (pH=7) was used to extract the exchangeable cations (Ca, Mg, K, and Na). Exchangeable Ca and Mg were measured by EDTA titrimetric method and exchangeable K and Na by Flame Emission Spectrophotometer (Sahlemedhin Sertsu and Taye Bekele, 2000).

Plant available K was determined by the ammonium acetate (CH_3COONH_4) method and the result was measured by a flame photometer (Bashour and Sayegh, 2007); whilst available P was extracted by sodium bicarbonate solution as described before (ISO., 1994) and thereafter followed by spectrometric measurement at 825 nm. Soil organic carbon (OC) and total N (TN) content were determined by dry combustion methods based on (ISO, 1998; ISO., 1995) respectively. Soil organic matter (OM) was calculated by multiplying soil organic carbon by 1.724 assuming an average C concentration of organic matter of 58%, (%OM = %OC x 1.724). Micronutrients (Cu, Fe, Mn, and Zn) were extracted with ammonium bicarbonate di-ethylene tri-amine Penta-acetic acid (DTPA), as described before (Tan, 1996). The concentrations were analyzed by flame atomic absorption spectrophotometer (FAAS). Relative percent of soil treated with OAs and that of the control was determined as:

$$\frac{\text{Treated}-\text{Control}}{\text{Control}} \text{ x } 100$$

3.2.6. Greenhouse experiments

The seeds of alfalfa and grass pea were surface sterilized with 3% sodium hypochlorite and 70% ethanol and washed several times with sterilized distilled water from which five seeds were sown at a depth of 2 cm separately into 3.5 Kg capacity plastic pots filled with 50:50 ratio of OAs and soil per pot followed by a reduction to three plants 10 days later. The seedlings were thinned down to three per pot after establishment (10 days). The experiment was arranged in five (5) treatments (Ts): T1- Biochar, T2- Compost, T3- Manure, T4- Biochar + Compost, T5-Biochar + Manure, T6-

Compost + Manure, T7- Biochar + Compost + Manure, and T8- Control (Soil only) using standard methods (Elouear *et al.*, 2016).

All pots were arranged in a randomized complete block design in triplicates in a greenhouse with 12 h photoperiod, day temperature (25±2 °C), and night temperature (17±3 °C). Each pot was watered daily with tap water for 60 days. Shoot height (SH), shoot dry weight (SDW), root dry weight (RDW), shoot fresh weight (SFW), nodule number (NN), nodule fresh weight (NFW), and nodule dry weight (NDW) were measured.

3.2.7. Statistical analysis

Mean separation was done using the Duncan multiple grouping of means at a 5% probability level when the ANOVA showed significant effects on soil physicochemical parameters after biochar and other organic amendments. Duncan's multiple grouping of means was used to separate the means. The values were presented as means ± standard deviation (SD), where $p \leq 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SAS software package (version 9.0).

3.3. Results

3.3.1. Effects of OAs on soil physicochemical properties

The amendments with biochar, compost, manure alone, or in combination had affected the physicochemical properties of soil differently compared to the control (Table 3.3). Soil pH was dramatically increased in BAS (8.4) with the application of the selected OAs ($p \leq 0.05$) compared to the control (5.69). Among the four amendments, BAS had the greatest impact on pH (8.4) followed by BCMAS (8.15). BAS, CAS, MAS, and BCMAS increased the soil pH by 2.71, 2.12, 2.26, and 2.46 units obtained by subtracting from the control, respectively. The present result indicated that the addition of OAs could increase soil pH. Soil pH increase was in the order of BAS > BCMAS >CAS > MAS compared to the control.

Compared to the control, OAs increased the total Nitrogen (TN) content in the soil. The largest increase in soil N was obtained with the biochar application (0.98%) followed by mixed (0.86%), compost (0.66%), and manure (0.55%) (Table 3.3).

The available P content in the soil increased in each amended soil. The highest available P was found in BCMAS (28.53 ppm) amended soil, whilst the highest available K contents were recorded from MAS (2.16 meq/100) after 12 months of the amendment (Table 3.3). The results show a significant increase in physicochemical properties in all the amended soil samples compared to the control ($p \leq 0.05$).

Table 3.3. The effect of the different OAs on degraded soil physicochemical properties (means ± standard deviation, n = 2)

Property	Unit	BAS	CAS	MAS	BCMAS	Total mean	Control	Sig.
Moisture	%	12.05b (0.92)	12.29b (0.72)	12.64ab (0.75)	15.15a (0. 38)	13.03	9.2c (1.41)	0.011
EC	dS/m	0.58c(0.03)	1.24b (0.17)	1.48ab (0.06)	1.24a (0.06)	1.14	0.25d (0.07)	0.0006
pH (H2O)	-	8.4a (0.33)	7.99a (0.15)	7.96a (0.37)	8.15a (0.55)	8.13	5.69b (0.16)	0.004
Av. P	Ppm	26.33a (0.51)	24.68ab (0.47)	25.71ab (0.74)	28.53a (1.07)	26.31	18.9b (2.96)	0.041
CEC	cmolc/Kg	53.55a (0.89)	46.86cd (0.61)	48. 69bc (0.62)	50.82ab (1.89)	49.98	43.78d (1.19)	0.002
Ex. Na	cmolc/Kg	0.59b (0.11)	0.44b (0.06)	0.89a (0.03)	0.97a (0.12)	0.72	0.18c (0.05)	0.001
Ex. K	cmolc/Kg	0.37a (0.06)	0.22b (0.04)	0.41a (0.03)	0.42a (0.05)	0.35	0.18b (0.06)	0.012
Ex. Ca	cmolc/Kg	27.66a (1.11)	33.37b (0.66)	30.06a (0.86)	31.65a (0.95)	30.68	25.26b (1.36)	0.003
Ex. Mg	cmolc/Kg	13.16c (0.56)	16.49b (0.93)	19.61a (0.98)	20.21a (1.24)	17.37	12.63c (1.17)	0.002
Av. K	meq/100 g	0.84b (0.33)	1.11b (0.18)	2.16a (0.09)	2.13a (0.22)	1.56	0.14c (0.05)	0.001
TN	%	0.98a (0.12)	0.66ab (0.13)	0.55b (0.11)	0.86ab (0.19)	0.76	0.13c (0.02)	0.007
C: N	-	14.93a (0.38)	11.85a (0.82)	13.9a (0.17)	13.32a (1.24)	13.5	10.92a (3.75)	0.314

BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD). Av = available, CEC= cation exchange capacity, Ex=exchangeable, EC = electrical conductivity, TN = total nitrogen, C: N = carbon to nitrogen ratio

The addition of biochar, compost, and manure increased Cu, Fe, Zn, and Mn concentration, as did the biochar + compost + manure treatments. The concentration of micronutrients was found to be in the order of Fe >Mn> Cu > Zn in almost all the amended soil of the study site (Figure 3.2). The corresponding values are 46.84, 46.90, 48.27, 48.77 and 42.50 for Fe, 6.41, 8.06, 8.41, 7.95, 5.85 for Mn, 3.14, 3.44, 4.25, 3.98, and 2.92 for Cu, 1.08, 1.91, 2.66, 2.30, and 0.58 mg/Kg for Zn in BAS, CAS, MAS, BCMAS and control plots.

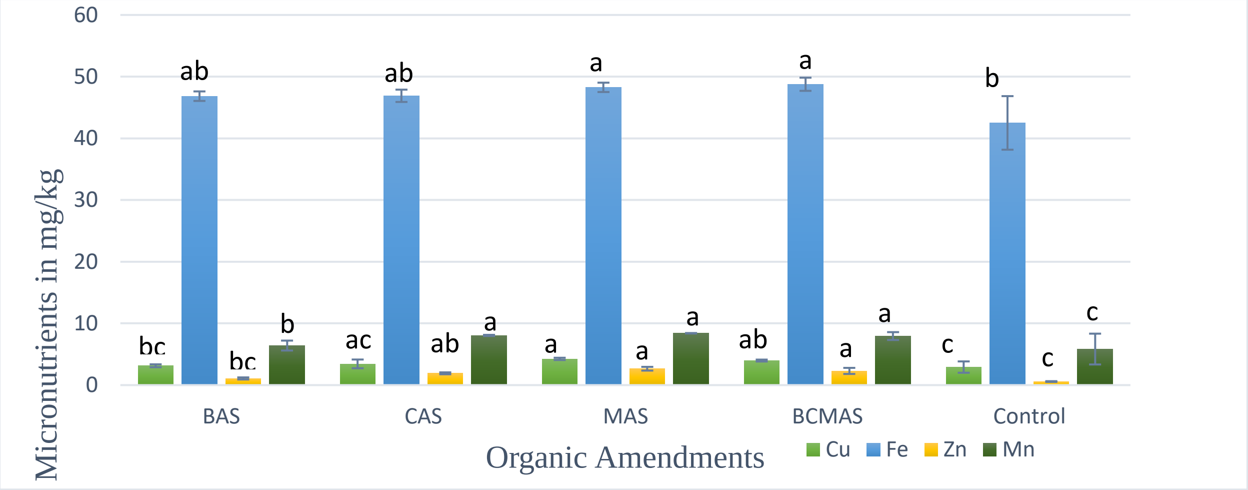

Figure 3.2. The effect of the different OAs on contents of micronutrients of the degraded soil.

BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD).

3.3.2. Effects of OAs on soil organic matter (OM) and Organic carbon (OC)

Soil OM significantly ($p \leq 0.05$) increased with the treatment of OAs were 2.69% in CAS and 4.86% in BCMAS compared to the control (2.43% Figure 3.3). The OM content increments were 2.28%, 0.26%, 0.96%, and 2.43% in biochar, compost, manure, and mixed treated soil, respectively. Similarly, OC was 1.63 and 3.075% in CAS and BCMAS, respectively compared to the control (1.405%; Figure 3.3).

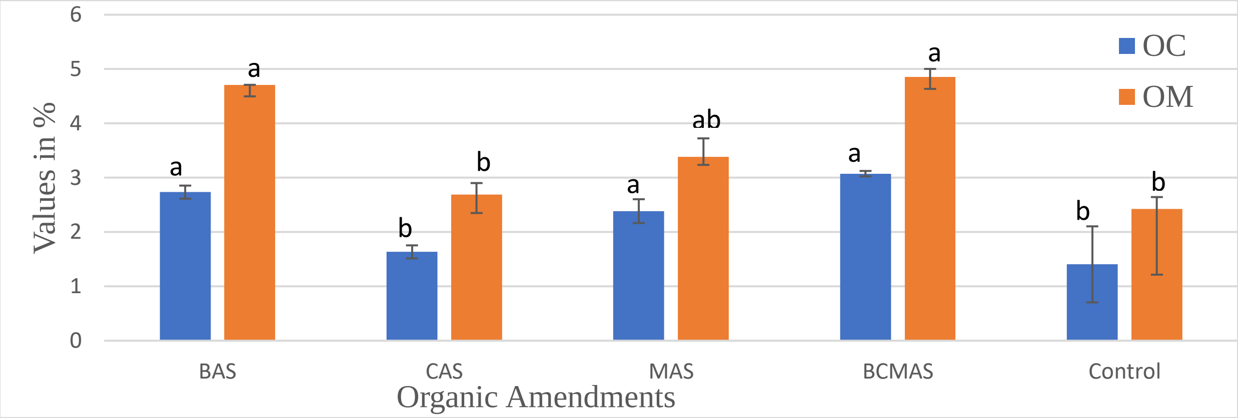

Figure 3.3. The status of OC and OM of the soil after organic amendments.

BAS= biochar amended soil. CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD). OC = organic carbon, OM = organic matter

3.3.3. Effects of OAs on CEC and exchangeable cations

In this study, soil CEC was higher in all amended plots than in the control and significantly ($p \leq 0.05$) different among the amended plots. Soil CEC was found to be higher in the biochar-amended plot (53.55 cmol$_c$/kg) followed by mixed amendments (50.82 cmol$_c$/kg) compared to control (43.78 cmo$_c$/kg) treatments (Table 3.3).

Compared to the control, the application of OAs alone or in combination increased the exchangeable Ca, Mg, K, and Na contents in the soil (Table 3.3). Compared to the biochar, the compost used had greater proportions of exchangeable Ca and Mg, 33.37, and 16.49 cmol_c/kg, respectively. The highest exchangeable K and Na content were found in the combined treatment (0.42, 0.97 cmol_c/kg, respectively), while the least was found in the control (0.18 cmol_c/kg) (Table 3.3). Among the OAs, biochar and compost had the lowest value in soil exchangeable Na^+ and Mg^{2+} compared to other amendments. If the soil turns into sodium saturated and forms Na-clay, the soil becomes less fertile. For soluble salts, the addition of OAs had a significant influence on K^+ (Table 3.3). In the present finding, the highest increase in exchangeable K^+ was observed in manure and combinations amended soils.

3.3.4. Effects of OAs on plant biomass under greenhouse

In the present study, the highest nodule number per plant was measured from BCMAS (118, 111/plant, respectively) in alfalfa and grass pea compared to the control (50, 58/plant, respectively; (Figure 3.4). Moreover, BAS had the higher nodule numbers (91 and 93) both in alfalfa and grass pea compared to CAS (77, 69) and MAS (84, 88) treatments both in alfalfa and grass pea, correspondingly. The OAs application significantly ($p \leq 0.05$) increased the biomass of alfalfa and grass pea. The significant increments were shown in response to the combined application of the amendments (Figure 3.5, Table 3.4, and 3.5). Among eight amendments, the triple treatments had the largest effect on the biomass, reaching 3.43 g fivefold of the control (0.7 g) in alfalfa and 4.54 g twofold of the control (2.07 g) in grass pea ($p \leq 0.05$). The effect of single and combined organic amendments on the growth of alfalfa and grass pea were indicated in (Figures 3.6 and 3.7) as compared to the control treatment. The figures realized that the combined application of biochar and other organic amendments showed better growth performance followed by a single application as compared to the non-amended soil.

Table 3.4. Growth performance of alfalfa on organic amended soils under greenhouse trials

Parameters	BAS	CAS	MAS	BCAS	BMAS	CMAS	BCMAS	CONT.	p-value
NFW	0.73b ± 0.09	0.83b ± 0.07	0.7b ± 0.17	0.86b ± 0.11	0.88b ± 0.02	0.86b ± 0.11	1.23a ± 0.12	0.49 c ± 0.07	0.00002
NDW	0.08 b ± 0.01	0.09 b ± 0.02	0.08b ± 0.03	0.1b ± 0.03	0.09b ± 0.03	0.12 b ± 0.08	0.29 a ± 0.07	0.05 b ± 0.01	0.00012
SH	72.67ab ± 10.78	60bcd ± 7.81	53 cd ± 12.77	69.67 abc ± 7.37	67 abc ± 4.36	53.33 cd ± 5.51	81.67 a ± 11.9	47 d ± 3.61	0.0023
SFW	6.30ab ± 1.58	10.14a ± 2.60	5.26 ab ± 1.47	8.29 a ± 0.39	8.74 a ± 6.77	9.59 a ± 0.75	10.27 a ± 2.88	2.46 b ± 0.24	0.038
RFW	0.69bc ± 0.19	1.33 a ± 0.55	0.38 c ± 0.14	0.58bc ± 0.07	0.29 c ± 0.16	0.76 abc ± 0.40	1.05 ab ± 0.42	0.28 c ± 0.09	0.0058
RDW	0.23 ab ± 0.06	0.25 a ± 0.10	0.26 a ± 0.07	0.22 ab ± 0.03	0.23 ab ± 0.11	0.29 a ± 0.13	0.35 a ± 0.09	0.1 b ± 0.03	0.0986

NFW- nodule fresh weight, NDW- nodule dry weight, SH- shoot height, SFH- shoot fresh weight, RFW- root fresh weight, RDW- root dry weight. BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD).

Table 3.5. Growth performance of grass pea on organic amended soils under greenhouse trials.

Parameters	BAS	CAS	MAS	BCAS	BMAS	CMAS	BCMAS	CONT.	P-value.
NFW	0.61 cd ±0.09	0.75bc ± 0.08	0.61 cd ± 0.14	0.83 b ± 0.09	0.77bc ± 0.09	0.83 b ± 0.15	1.11 a ± 0.14	0.45 d ± 0.07	0.00011
NDW	0.07 b ± 0.01	0.08 b ± 0.02	0.07 b ± 0.03	0.12 b ± 0.07	0.07 b ± 0.03	0.12 b ± 0.09	0.28 a ± 0.07	0.04 ab ± 0.02	0.0007
SH	83.67 ab ± 3.78	96.33 a ± 2.89	96 a ± 11.27	91.67 a ± 10.6	98 a ± 28.67	96 a ± 19.08	107.67 a ± 10.02	65.67 b ± 4.04	0.069
SFW	6.08bc ± 0.66	5.53 c ± 2.20	6.99abc ± 2.09	7.44 abc ± 1.23	8.31 ab ± 0.49	8.89 a ± 1.05	9.64 a ± 1.37	2.89 d ± 0.56	0.00042
RFW	0.15 b ± 0.05	0.14 b ± 0.01	0.13 b ± 0.03	0.17 b ± 0.05	0.19 b ± 0.06	0.16 b ± 0.04	0.4 a ± 0.24	0.11 b ± 0.002	0.0333
RDW	0.07 b ± 0.02	0.06 b ± 0.03	0.07 b ± 0.02	0.08 ab ± 0.02	0.08 ab ± 0.02	0.05 b ± 0.02	0.12 a ± 0.05	0.03 b ± 0.02	0.0265

BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD). NFW- nodule fresh weight, NDW- nodule dry weight, SH- shoot height, SFH- shoot fresh weight, RFW- root fresh weight, RDW- root dry weight

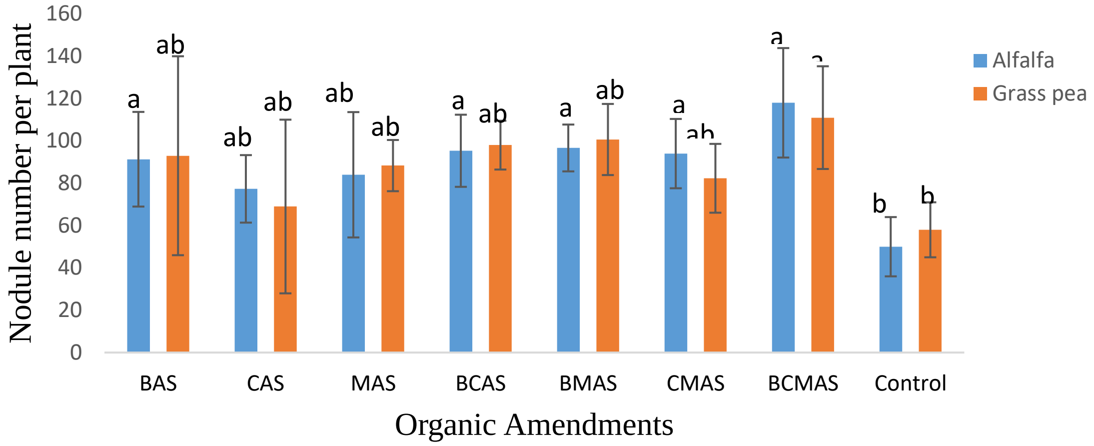

Figure 3.4. Comparison of nodule numbers per plant of alfalfa and grass pea on organically amended soil.

BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD).

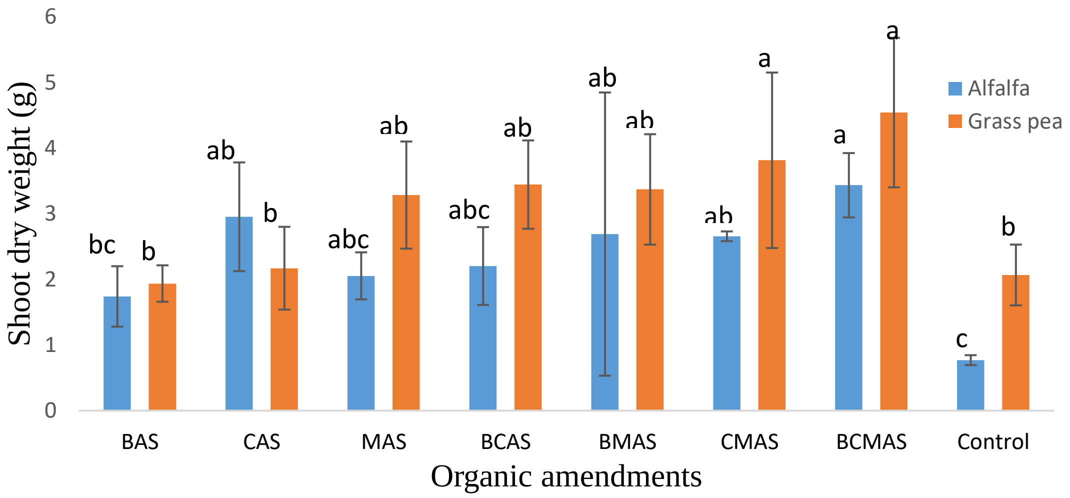

Figure 3.5. The effect of OAs on the shoot dry weight of alfalfa and grass pea

BAS= biochar amended soil, CAS = Compost amended soil, MAS = manure amended soil, BCMAS = biochar + compost + manure amended soil. Means with the same letter are not significantly different at $p \leq 0.05$ with Duncan grouping using (mean ± SD).

Single amendments
Combined amendments
Control treatment

Figure 3.6. The effect of single and combined organic amendments on the growth of alfalfa crop compared to the control

Combined amendments
Single amendment
Control treatment

Figure 3.7.The effect of single and combined OAs on the growth of grass pea crop compared to the control

3.4. Discussion

Soil pH is one of the major factors that affect soil chemical processes. The data showed an increase in soil pH due to the sole application of biochar. This could be attributed to the high pH of the biochar that contains a large amount of ash with alkaline. The change in soil acidity following OAs as compared to the control is due to the presence of organic matter that can increase the CEC of the soil. Hence, as the base saturation increases, the relative amount of acid cations decreases.

Similarly, Silva *et al.* (2017) showed the application of biochar from rice husk, sorghum silage, and sawdust increased the soil pH by 0.76, 1.17, and 1.68 units, respectively due to biochar amendment. The application of biosolids such as animal manure and compost on acid soils increases the soil pH from 5.16 to 7.19 (Odoemelam and Ajunwa, 2017).

There are also other explanations for changes in pH that include decarboxylation of organic anions due to decomposition, complexation of free H^+ and Al^{3+} ions with organic ligands, and increased saturation of soil CEC by Ca^{2+}, Mg^{2+} Na^+, and K^+ added by the wastes (Pavinato and Rosolem, 2008). The increase in TN of the soil after amendments is closely related to the build-up of organic matter in the soil (Table 3.3). Similarly, Saldaña *et al.* (2014) reported that the increased TN content with manure (0.36%), liquid humus (0.31%), and compost (0.31%) amendments. Dodor *et al.* (2018) also showed a TN increase due to the application of biochar (1.6 g/kg) and cattle manure (11.6 g/kg) compared to the control group (0.18 g/kg). Furthermore, it had been reported that the addition of organic manures (cow, sheep, and poultry) increased net N released by 42, 25, and 43%, respectively over the control (Abbasi *et al.*, 2007). The increase of P and K availabilities (adsorption) in organic amendments might be due to the competition of these cations with Fe and Al ions to the negative charges of the functional groups of the organic matter in the soil solution (Reynolds *et al.* (2015). The authors also showed the application of OA also induces high microbial activity. Nziguheba *et al.* (1998) have indicated that OAs may increase P availability, either directly from the decomposition of organic matter and release of P or indirectly by increasing the amount of soluble organic acids that increase the rate of desorption of phosphate. Melero *et al.* (2007) have also observed that increase in high microbial activity induced by the addition of amendments which increased P cycling. Moreover, P can be adsorbed by soil colloids, and the mixed OAs can bind large quantities of macronutrients and thereby reduce their removal from soil by leaching (Withers *et al.*, 2003). Walker and Bernal (2008) observed poultry that the manure and compost treatments resulted in significantly increased soil soluble K^+ probably related to the application of OAs.

The micronutrient availability was more pronounced in the combined amendments which might be due to their synergistic activities (Mohhammed *et al.*, 2016). Similarly, soil OAs with biochar, compost, and cattle manure significantly increased the availability of these trace elements in the range of 6.1- 460 mg/kg Carmo *et al.* (2016), as compared to 1.08 - 48.77 mg/kg of this finding.

The present study indicated that the OAs significantly increased the OM and OC contents. The increase was more pronounced in biochar amended soil (Figure 3.3). Soil OM contents of < 2.0% is low; 2.1-3.0% is medium and > 3.1% as high as reported by (Odoemelam and Ajunwa, 2017). Maltas *et al.* (2018) and Trivedi *et al.* (2017) have also indicated that organic amendments supplied 25 to 80 % additional C input to the soil. Tejada *et al.* (2006) concluded that the chemical property of the OAs decided the effect of amendments on soil OM and OC. Application of farmyard manure visibly enhanced the soil OC content in various cropping systems (Iqbal *et al.*, 2012).

Cation exchange capacity provides buffering against changes in pH, available nutrients, calcium levels, and soil structure. The reason for high CEC in biochar amended plot is partly due to an increase in the surface area and charge density on the surface. Nguyen *et al.* (2018) demonstrated that biochar resulted in higher soil CEC (29 $\text{cmol}_c\ \text{kg}^{-1}$) than the control (25.6 $\text{cmol}_c\ \text{kg}^{-1}$). Biochar from woody materials typically enhances the pH, soil water relations, and CEC and ultimately results in improved soil fertility (Uzoma *et al.*, 2011). The soil water-holding capacity (WHC) of 20% biochar amendment soil was 1.9 times as pure as sandy soil. In addition, the incorporation of biochar did not only ameliorate soil acidity at the beginning but also increased soil pH buffering capacity, providing suitable soil pH a few months after application (Chang *et al.*, 2021). Cheng *et al.* (2008) reported that the addition of biochar during one year raised its CEC from 1.7 to 71 mmol/ kg.

Applications of composts and manures increase soil fertility and the increase in the short term since the added organic matter is quickly oxidized to add bases that rapidly leached (Tiessen *et al.*, 1994). On the contrary, the application of biochar to infertile soil provides long-lasting improvements in soil fertility (Steiner *et al.*, 2007). Likewise, Chan *et al.* (2008) reported biochar input promotes positive changes in soil quality, such as acidity correction, increased CEC. These help greater nutrients use efficiency and absorption by roots of plants.

The applied OAs increased exchangeable Ca^{2+} contents of soil, which may increase the replacement of Na^+ from the exchange sites, thus improving the remediation efficiency of soil (Table 3.3). Compost had greater proportions of exchangeable Ca and Mg, 162.7, and 22.7 $cmol_c\ kg^{-1}$, respectively (Mensah and Frimpong, 2018). The increase in the exchangeable bases is a result of the presence of ash in the biochar helps in the immediate release of mineral nutrients like Ca and K for crop use (Abebe Nigussie *et al.*, 2012).

Alfalfa and grass pea plants receiving biochar, manure, or compost produced more aerial fresh and dry biomass. Plants amended with triple combination treatments produced 25% and 29% higher fresh biomass in alfalfa and grass pea, respectively. The basic reason for the higher symbiotic nitrogen fixation and root nodule numbers in legumes is attributed to the increased availability of the trace nutrients such as boron and molybdenum in biochar. The high C: N ratio of biochar in BAS might favor the growth of free-living and plant-associated nitrogen-fixing microorganisms.

Moreover, many organic wastes are a source of N, P, Ca, and other elements such as Zn, Cu, and Mg that are essential to plant growth. A similar study from the greenhouse experiment confirmed that biochar increased nodule biomass and numbers in legume plants (Güereña *et al.*, 2015). Mia *et al.* (2014) demonstrated that biochar considerably increased the number of plant root nodules in red clover (*Trifolium pratense* L.). Benabderrahim *et al.* (2018) indicated an increase of 21 and 19% fresh biomass of alfalfa in compost and manure-amended soil, respectively. In this study, application of compost increase root fresh weight. This might be due to the immediate nutrient release and water holding capacity. Stuckey and Hudak (2001) reported that, the application of large amounts of compost (62 ton/ha, filled into the planting holes) doubled the survival rate of *Pinus taeda* L. and increased growth by 41%.

The dry weight of the plants significantly increased by 1.31, 1.37, and 1.49 folds with the treatment of rapeseed meal, manure, and biochar-amended soil compared to the control in wheat biomass (Yang *et al.*; Yang *et al.*, 2018).

3.5. Conclusion

This study revealed that the application of biochar, compost, manure alone, or in combination significantly increased soil pH, total OC, OM, available phosphorus, total nitrogen, CEC, and micronutrients in soils. Moreover, mixed application and a single application of biochar, compost, and manure increased the plant height, root length, dry and fresh matter, nodule numbers in cover crops (alfalfa and grass pea) under greenhouse conditions. The application of biochar and other organic amendments can recover soil fertility of degraded land. This study suggests that the utilization of easily available bio-waste for degraded land reestablishment to benefit the ecosystem and the community.

Chapter 4

4. Genetic and Metabolic Diversities of Rhizobacteria Isolated from Degraded Soil of Ethiopia

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Abstract

Genetic and metabolic properties of rhizobacteria are the fundamental sources for their adaptation to cope with abiotic and biotic stresses and enhance the growth and health of plants in the soil. Thus, this study was initiated to assess the genetic and metabolic diversities of rhizobacteria isolated from plants grown in degraded soil through BOX-PCR and partial sequencing of 16S rRNA genes. A total of 73 isolates were recovered and subjected to phenotypic profiling of carbohydrate and amino acid utilization, BOX PCR, and 16S rRNA profiling. The phenotypic profiling showed that metabolic versatility of 14.29 to 100%. Thus, *Ochrobactrum* spp, *Pseudomonas* spp, and *Klebsiella* spp expressed remarkable metabolic versatility, and BOX-PCR showed greater discriminatory power for fingerprinting of rhizobacterial isolates with a high degree of polymorphism. *Bacillus* spp showed the highest Simpson's diversity index of 0.76 of all the isolates; the 16S rRNA genes sequence assigned the rhizobacteria to phyla Proteobacteria (78.08%) with classes Gammaproteobacteria (56.16%) and Alphaproteobacteria (21.92%). It also showed that the phylum Firmicutes contributed to (21.92%) of the taxon of which the class Bacilli (21.92%). The data also showed that the most dominant species were *Pseudomonas* (23%) followed by *Ochrobactrum* (21%). Genetic and metabolic diversities of the rhizobacterial isolates reveal the potential of these microbes for plant growth improvement under water-deficient soil after testing other inoculant traits.

Keywords: BOX PCR, 16S rRNA genes, genomic diversity, nutritional profile, PGPR

4.1. Introduction

Soil is considered a rich reservoir of diverse groups of microorganisms involved in the biogeochemical cycles, and untapped resources for agricultural and industrial applications (Mhete *et al.*, 2020). The rhizosphere of plants is the hot spot of microbial activities dominated by bacteria generally known as rhizobacteria. The rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed as plant growth-promoting rhizobacteria (PGPR) (Schroth and Kloepper, 1978).

Metabolic diversity of rhizobacteria is reduced through intensive land-use, which may have implications for the resistance of the soils to stress or disturbance (Ding *et al.*, 2013). This results in losses of characteristic flora and fauna, and may also affect microbial diversity. Hence, need the selection and exploitation of rhizobacteria for restoration to improve soil fertility, maintain ecological balance and environmental quality (Zahid, 2015).

The rhizobacteria enhance plant growth by improving nutrient availability, increasing nutrient uptake, enhance plant resistance to biotic and abiotic stresses (Mesa *et al.*, 2015). A diverse array of rhizobacteria is used for maintaining soil fertility that includes *Azospirillum*, *Bacillus*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Klebsiella*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Enterococcus* (Solanki *et al.*, 2017; Xing *et al.*, 2016).

A wide-ranging evaluation of genetic and metabolic diversities can be useful for the introduction of new and useful microorganisms into the environment (Joseph *et al.*, 2012). The metabolic assets of an organism could contribute towards a particular environmental adaptation (Mazur *et al.*, 2013).

A significant number of studies have been focused on the isolation and identification of microbes by employing traditional physiological and biochemical methods (Liu *et al.*, 2006). Recently, molecular methods have been applied as the smartest means to investigate species diversity. Polymerase chain reaction-based methods such as BOX-PCR and analysis of 16S rRNA genes are appropriate tools to examine microbial diversity in a wider range of environments (Fakruddin *et al.*, 2013; Srinivasan *et al.*, 2015). Nowadays, bacterial classification involves techniques to determine both phenotypic and genotypic characteristics (polyphasic approach).

There is a clear incentive to exploit this microbial diversity to develop functional microbes that could be used as a targeted bio-tools to boost soil fertility. Thus, the main purpose of this study was to assess the genetic and metabolic diversities of culturable indigenous soil bacteria from the rhizosphere of degraded soil grown woody plants.

4.2. Materials and Methods

4.2.1. Rhizobacteria Isolations and Selection

Rhizosphere soil samples were taken from the rhizosphere of *Acacia abyssinica* and *Junipers procera* grown on degraded soils. The roots were shaken carefully inside plastic bags to separate the soil from the roots. Rhizobacteria were isolated from different sampling sites Fiche areas, Oromia National Regional State, Ethiopia and purified using standard methods Wang *et al.* (2021), and maintained in culture collection at Addis Ababa University. Totally, 250 rhizobacterial isolates were identified and were reduced to 80 based on drought tolerance potentials. They were screened for phenotypic carbohydrate and amino acid profiling and genetic characterization. But seven (7) isolates come up with poor sequences and the number of isolates is reduced to seventy-three (73).

4.2.2. The phenotypic profiling of carbohydrate and amino acid utilization

The nutritional versatility of the potential rhizobacteria isolates was assessed by their ability to utilize 15 carbohydrate and 7 amino acid sources. Growths of the isolates were checked for each microbe on the basal mineral salt medium (MSM) constructed for the tests of carbohydrates and amino acids utilization according to (Zajic and Supplisson, 1972). The carbon sources were adjusted to a final concentration of 1 g/L to a basal medium containing (per liter of distilled water: 1.8 g K_2HPO_4, 4.0

g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$, 15 g agar. The amino acids were added at a concentration of 0.5 g/L to the same basal medium from which NH_4Cl was omitted adjusted to pH 6. 9 (Amarger *et al.*, 1997). All of the substrates were filter sterilized using a membrane (pore size 0.45 µM, Millipore). The test rhizobacteria were grown overnight in nutrient broth from which 50 µL of culture was streaked on the MSM agar plates and incubated at 30ºC for 72 h. The results were recorded as (+) for growth or (-) for no growth in comparison with the controls. All the experiments were performed in triplicates.

4.2.3. Genotypic characterization

To prepare 48 µL of PCR mixture, 1.5 µL of both forward and reverse primers, 2 µL of DNA template, 1.5 µL of 10× Taq PCR buffer, 0.2 µL dNTPs, 1.5 mM $MgCl_2$, and 1.5 unit of Taq DNA polymerase were mixed together. The genotypic characterization was done via 16S rRNA and BOX-PCR fingerprinting (Ribeiro and Cardoso, 2012; Xavier *et al.,* 2017).

4.2.3.1 Genomic DNA extraction

Extraction of total genomic DNA from all the selected bacterial strains was performed using the DNeasy Tissue kit (Qiagen, Germany) according to manufacturer's instructions. Extracted DNA from pure cultures was used for 16S rRNA genes amplification using a universal primer pair for forward and reverse (Table 4.1). PCR products were purified with a QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The amplified product was sequenced by Sanger sequencing platform using capillary sequencer analyzer (Applied Biosystems, Brazil) (Paulitsch *et al.*, 2019). The PCR condition is presented in Table 4.2.

Table 4.1. PCR conditions for BOX-PCR and 16S rRNA

Target gene	Primer	Sequence (5′→ 3′)	Product size	References
16S	Forward (fD1)	5′-AGAGTTTGATCCTGGCTCAG-3′	1100-1300	(Weisburg *et al.* 1991)
	Reverse (rD1)	5′-AAGGAGGTGATC CAGCC-3′		
BOX	BOXA1R	5’CTACGGCAAGGCGACGCTGACG-3’	50-5000	(Guiñazú *et al.* 2013)

4.2.3.2. Genetic diversity BOX-PCR Fingerprinting

In BOX-PCR genomic fingerprint, BOXA1R primer was used (Table 4.2). To prepare 25 µL of PCR mixture, 1 µL primers, 2 µL of DNA template, 2.5 µL Taq PCR buffer, 5 µL dNTPs, 1.5 µL $MgCl_2$, and 0.2 U Taq DNA polymerase (Promega) were mixed. The PCR reaction was carried out according to the condition in Table 4.1. The PCR products were separated in 1.5% agarose gel with 1 kb DNA ladder (Invitrogen). Then, the gel was stained with ethidium bromide and viewed under a UV transilluminator (Loccus, Brazil).

The DNA band patterns were analyzed and a dendrogram was generated for each isolate by using Bionumerics 7.3 software program (Applied Mathematics, Brazil) by applying the UPGMA algorithm (Unweighted Pair-Group Method with Arithmetic mean) and the Jaccard’s coefficient with 3% of tolerance (Sneath and Sokal, 1973). Differences among strains were assessed visually based on the banding patterns of PCR products.

Simpson's Index of Diversity, D, was also calculated. The discriminating power of this typing method was calculated by using Simpson's Index of Diversity, D (Hunter and Gaston, 1988). The higher the discriminatory index, the greater the effectiveness of a particular fingerprinting method to discriminate different strains (Yoke-Kqueen *et al.*, 2013). This index was given by the following equation:

$$D = 1 - \frac{\sum ni(ni-1)}{N(N-1)}$$

Where N is the total number of strains in the sample population, ni denotes the number of strains belonging to the ith type.

Table 4.2. PCR conditions for BOX-PCR and 16S rRNA

	BOX-PCR		
PCR steps	Temperature (°C)	Duration (min/sec)	Cycle
Initial denaturation	95	7'	-
Denaturation	94	1'	
Annealing	53	1'	
Elongation	65	8'	30
Final elongation	65	16'	-
	16S rRNA		
Initial denaturation	95	2'	
Denaturation	94	15''	
Annealing	55	45''	30
Elongation	72	2'	
Final elongation	72	5'	

Sequences and accession numbers were deposited in the Gene Bank database (NCBI) and received accession numbers MN005961-MN006030 for 16S rRNA sequences. The accession numbers are listed in parentheses in the phylogenetic trees. Four phylogenetic trees were constructed for both Gram-positive and Gram-negative rhizobacterial strains.

4.2.4. Statistical Data Analysis

The carbohydrates and amino acids utilization pattern of rhizobacterial isolates was tabulated using percentages. BOX-PCR dendrogram was established using Bionumerics software (v.7.0.2) (Sneath and Sokal, 1973). All phylogenetic analyses were performed with the software MEGA 7 (Tamura *et al.*, 2013). Pairwise and multiple sequence alignments were generated with Clustal W (Larkin *et al.*, 2007). Tamura 3-parameter model Tamura *et al.* (2013) with G+I Tamura (1992) was used to determine the 16S rRNA phylogenies.

4.3. Results

6.3.1. Carbohydrates and Amino acids Utilization

A total of 73 isolates were tested for phenotypic profiling of carbohydrate and amino acid utilization patterns. All isolates were diversified into nine genera; *Ochrobactrum*, (28% of the isolates) *Enterococcus* (14%), *Klebsiella* (14%), *Pseudomonas* (14%), *Serratia* (10%), *Bacillus* (5%), *Morganella* (5%), *Paenibacillus* (5%), and *Agrobacterium* (5%) (Table 4.3).

Isolates utilized 15 of the carbohydrates tested (100%) (data not shown); whereas only 36.36% of the isolates utilized all the nitrogen sources (Table 4.3), indicating that they were more versatile to utilize carbohydrates than they were to nitrogen sources. Among representative isolates from six genera; *Enterococcus* PS-4, *Ochrobactrum* RS-70, *Ochrobactrum* RS-76, *Ochrobactrum* RS-77, *Agrobacterium* RS-79, *Pseudomonas* FB-49, *Klebsiella* PS-2, and *Serratia* RS-73 utilized all the tested carbohydrate and nitrogen sources indicating the dominance of *Ochrobactrum* in substrate utilization.

Table 4.3. Amino acids utilization patterns of some selected rhizobacterial isolates

		Amino acid sources							
S. No.	**Strains**	Asparagine	Arginine	Valine	Isoleucine	Serine	Tryptophan	Glycine	**Total (%)**
1	*Enterococcus* PS-4	+	+	+	+	+	+	+	100
2	*Agrobacterium* RS-79	+	+	+	+	+	+	+	100
3	*Ochrobactrum* RS-70	+	+	+	+	+	+	+	100
4	*Ochrobactrum* RS-76	+	+	+	+	+	+	+	100
5	*Ochrobactrum* RS-77	+	+	+	+	+	+	+	100
6	*Pseudomonas* FB-49	+	+	+	+	+	+	+	100
7	*Klebsiella* PS-2	+	+	+	+	+	+	+	100
8	*Serratia* RS-73	+	+	+	+	+	+	+	100
9	*Bacillus* BS-47	+	+	+	+	+	-	-	71.43
10	*Enterococcus* PS-5	+	+	+	-	-	-	+	57.14
11	*Enterococcus* PS-9	+	+	+	+	+	-	+	85.71
12	*Paenibacillus* FB-50	+	-	-	+	+	-	+	57.14

13	*Ochrobactrum* RS-58	+	+	+	+	+	-	+	85.71
14	*Ochrobactrum* RS-59	+	+	-	+	+	-	-	57.14
15	*Ochrobactrum* RS-68	+	+	+	+	+	-	+	85.71
16	*Ochrobactrum* RS-72	+	+	+	+	+	+	-	85.71
17	*Pseudomonas* BS-52	+	+	+	+	+	-	+	85.71
18	*Pseudomonas* BS-41	+	+	+	+	+	-	+	85.71
19	*Klebsiella* PS-1	+	+	+	+	+	+	-	85.71
20	*Klebsiella* PS-3	+	+	+	+	+	-	+	85.71
21	*Morganella* PS-13	+	+	+	+	+	+	-	85.71
22	*Serratia* PS-54	+	+	+	+	+	-	+	85.71

"+" and "–" = show bacterial growth on Nitrogen supplemented sources and no bacterial growth on N supplemented sources respectively

4.3.2. Genotypic Diversity

The BOX PCR fingerprint showed a significant genetic diversity of the rhizobacterial isolates (Figure 4.1). The dominance pattern was different from phenotypic profiling based on C and N utilization. Thus, *Bacillus* species showed the highest diversity (D = 0.762) followed by *Paenibacillus*, *Klebsiella*, and *Pantoea* with D = 0.667 and *Serratia* (D = 0.607) values. The overall Simpson's Index of the diversity of the current study indicated a greater bacterial diversity (D = 0.883; Table 4.4).

Table 4.4. The Simpson genetic diversity index (D) of rhizobacterial isolates from degraded soil

Genus	Number (n)	n(n-1)	D	Percent (%)
Bacillus	7	42	0.762	76
Enterococcus	6	30	0	NA
Paenibacillus	3	6	0.667	67
Agrobacterium	1	0	NA	NA
Ochrobactrum	15	210	0.133	13
Acinetobacter	1	0	NA	NA
Pseudomonas	17	272	0.426	43
Klebsiella	3	6	0.667	67
Morganella	9	72	0	NA
Pantoea	3	6	0.667	67
Serratia	8	56	0.607	61
Unidentified	7	42	NA	NA
Total (N)	**80**	**470**	**0.883**	**88**

NA = not applicable

4.3.3. BOX-PCR Fingerprinting

The dendrogram displaying the distance relationships between the strains is shown in Figure 4.1. At a distance of 0.70, eleven clusters were shown (I to XI). The banding pattern of BOX-PCR amplification yielded 5-24 bands (Figure 4.1). BS-35 strain displayed the highest number of bands (n=24), while BS-28 strain showed the lowest number of bands (n=5). Strains RS-70/RS-74, RS-72/RS-77, RS-60/RS-61, and BS-24/BS-41 had identical profiles. Moreover, strains BS-43/BS-53/BS-45 showed an identical profile. On the other hand, the largest number of strains; PS-10, RS-79, PS-55, BS-46, BS-27, PS-34, BS-31, BS-7, PS-13, BS-30, and BS-28 exhibited unique BOX PCR genomic profiles.

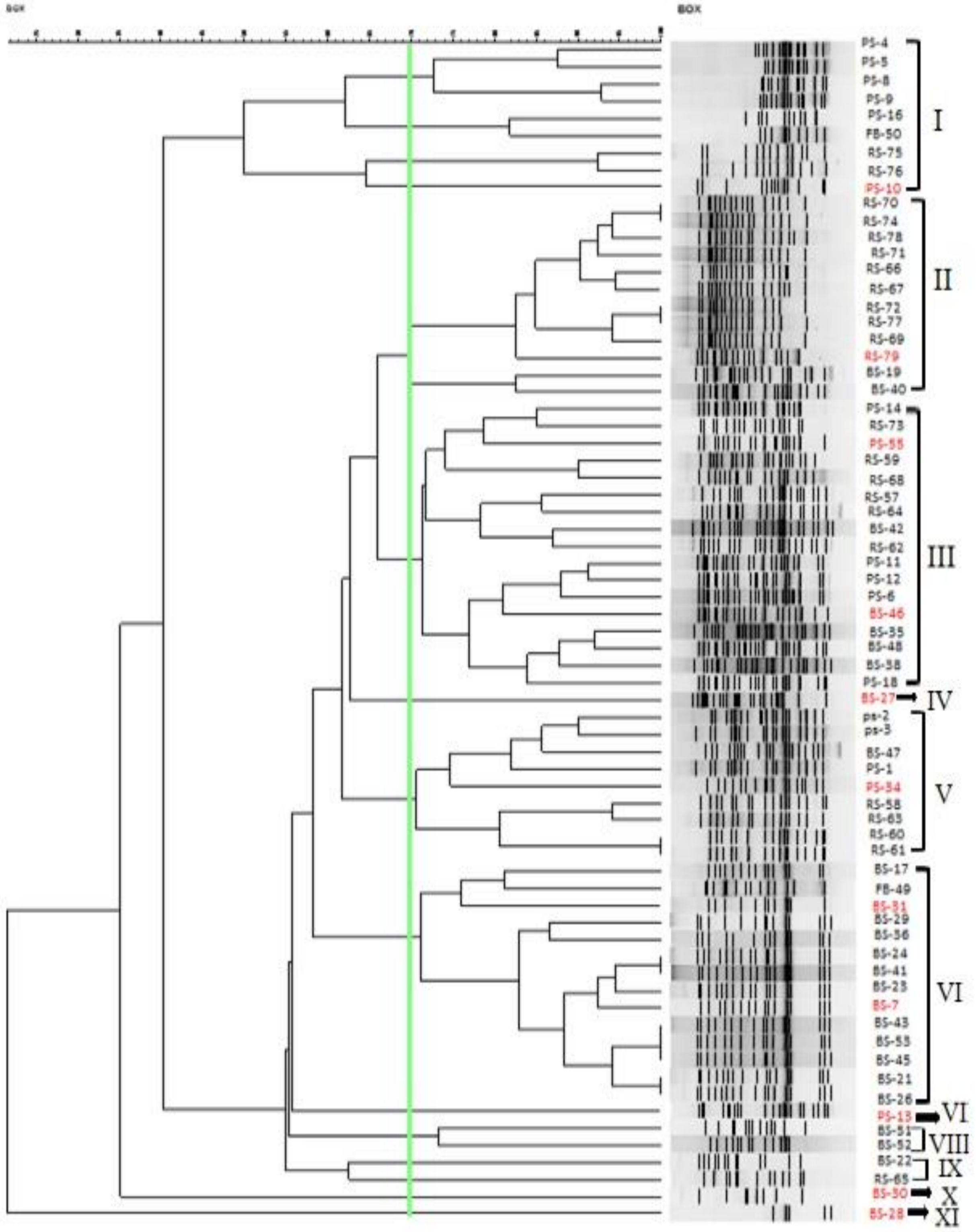

Figure 4.1. Dendrogram of similarity based on BOX-PCR profiles of rhizobacterial isolates those strains marked in red indicate unique genomic profiles

4.3.4. The 16S rRNA phylogeny of the rhizobacteria

In this study, the 16S rRNA sequence confirmed that Proteobacteria (78.08%) and Firmicutes (21.92%) were the dominant bacterial phyla isolated from the study site. The two phyla belonged to three major taxonomic classes, namely, Alphaproteobacteria, Bacilli, and Gammaproteobacteria where the latter was most dominant (56.16%) of the other groups (21.92% each) (Figure 4.2).

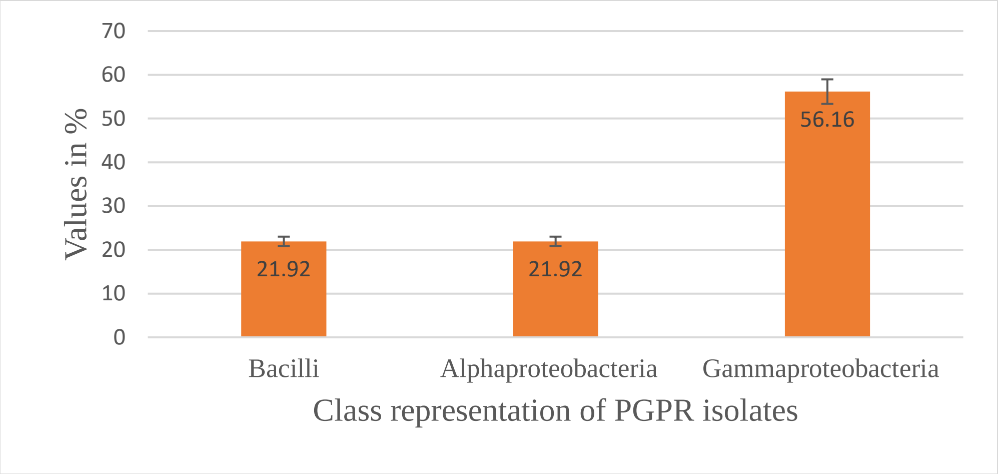

Figure 4.2. A class representation of each PGPR isolated from degraded land

The genotypic relationships of the gram-positive genera with closely related species are indicated in Figure 4.3.

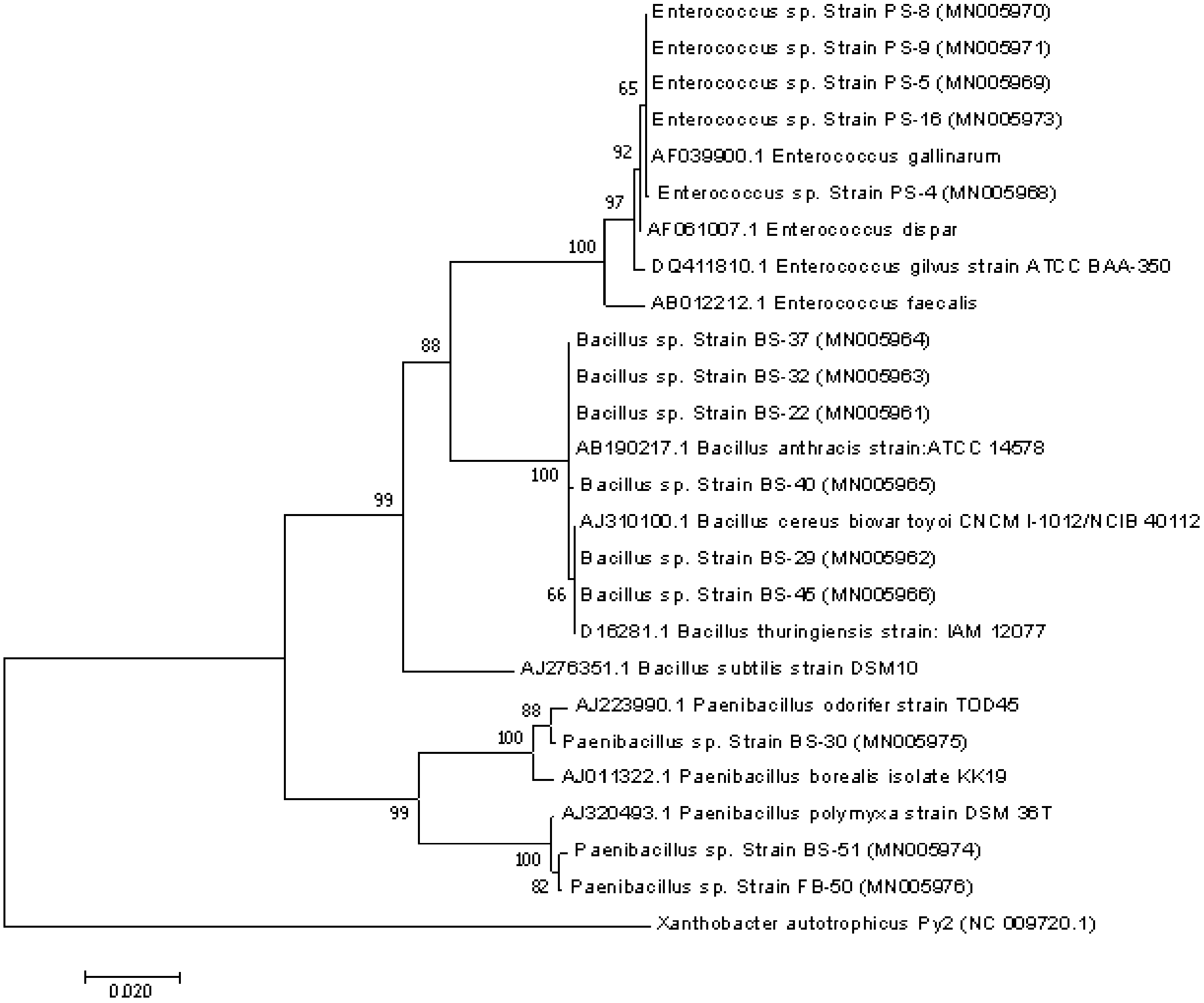

Figure 4.3. Phylogenetic tree of 16S rRNA gene sequences of Gram-positive rhizobacteria from degraded soil and some of their closest phylogenetic relatives using the Neighbor-Joining method.

The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Xanthobacter autotrophics* Py2 (NC-009720.1) species was used for out grouping

The genus *Agrobacterium* with 100% similarity with *A. tumefaciens*, and *Ochrobactrum* with 100% similarity (Figure 4.4). Sequences of the isolates affiliated to *O. intermedium* (n = 6) were more polymorphic with 100 % identity, while isolates belonged to *O. ciceri* (n = 8) had a 100% similarity. Sequence similarity among *O. anthropi*, *O. ciceri* and *O. intermedium* was 98% (Figure 4.4).

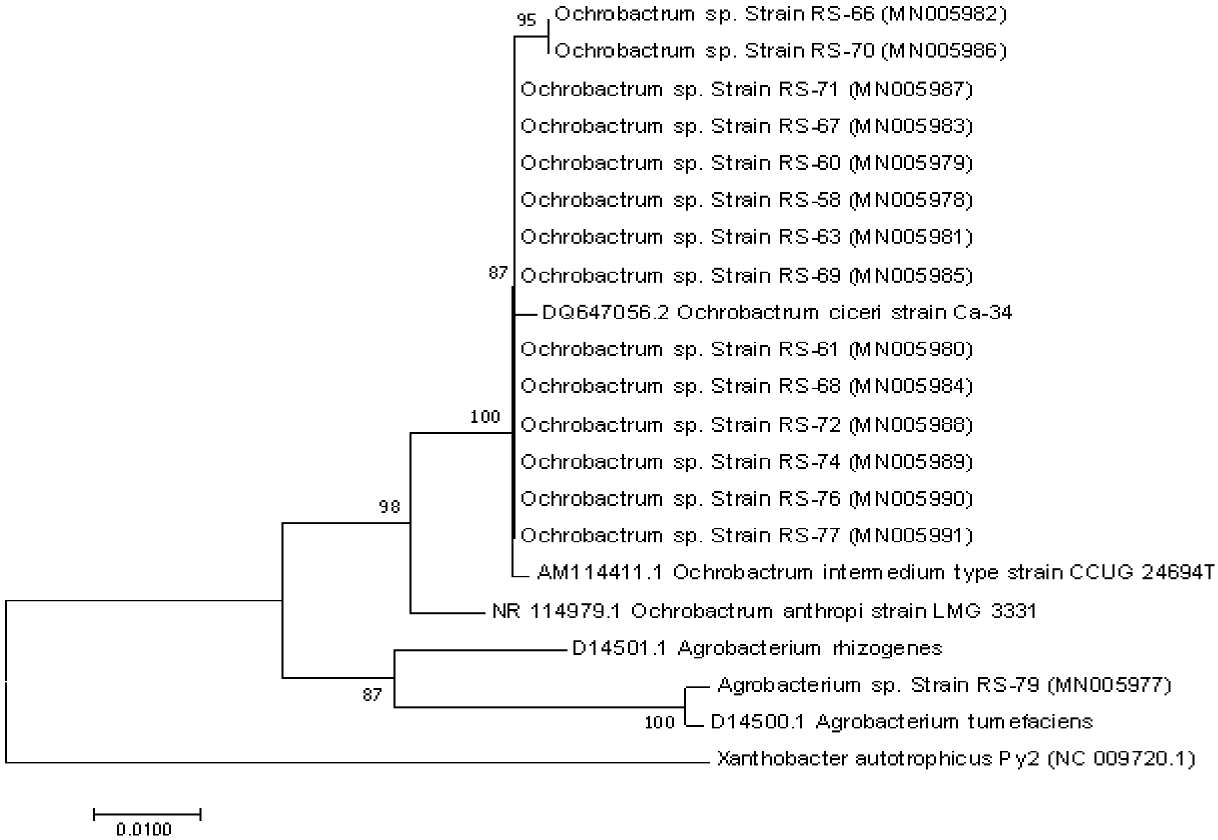

Figure 4.4. Phylogenetic tree of 16S rRNA gene sequences of Gram-negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method.

The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Xanthobacter autotrophics* Py2 (NC-009720.1) species was used for out grouping

In this study, *Pseudomonas* showed the most dominant genus (Figure 4.5). Strain BS-19 was grouped as *Pseudomonas fulva* with 96% similarity, while the majority of the strains were classified under *Pseudomonas para fulva* with 96% identity. Moreover, strain BS-26 fell under *Pseudomonas putida* with 96% similarity, while the strains FB-49 and RS-75 showed 98% similarity with *Pseudomonas fluorescens*. The strain BS-27 was another single genus of which had 99% similarity with *Acinetobacter calcoaceticus* (Figure 4.5).

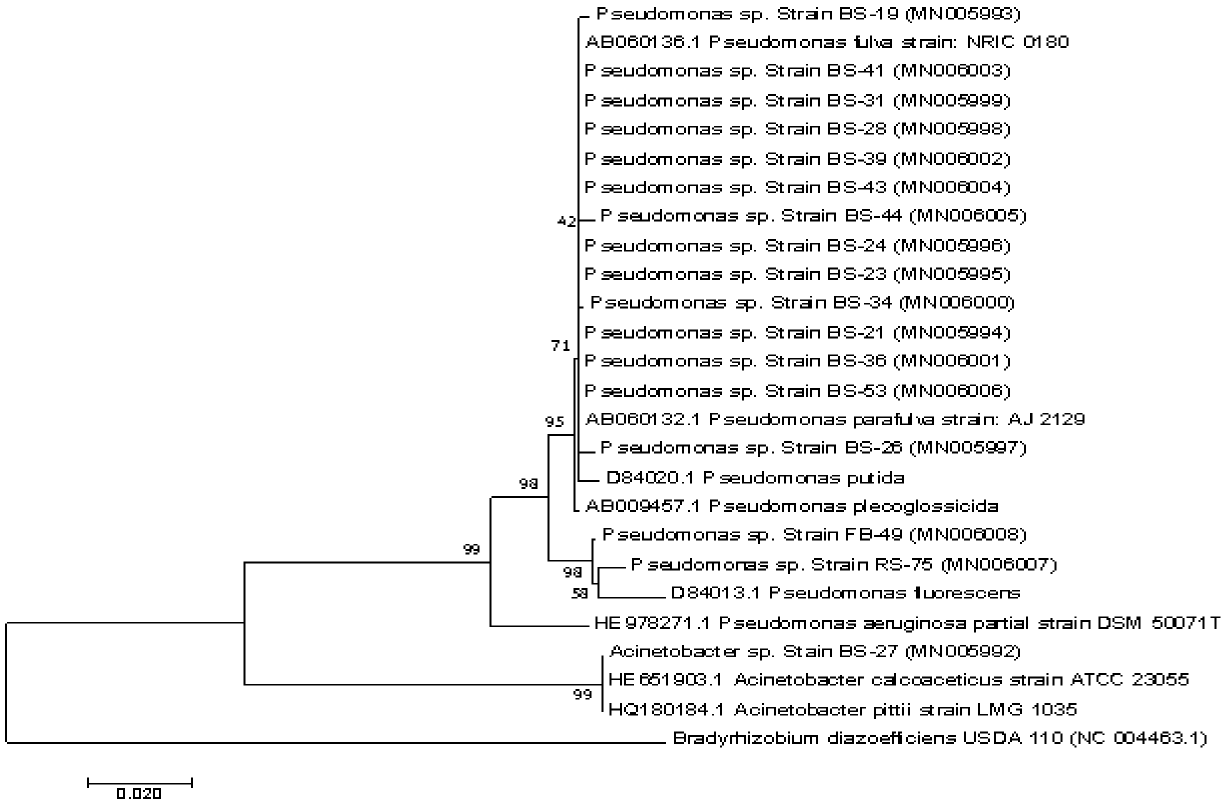

Figure 4.5. Phylogenetic tree of 16S rRNA gene sequences of Gram-negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method.

The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Bradyrhizobium diazoeficiens* USDA 110 species was used for out grouping

The genus *Morganella*, *Serratia*, *Klebsiella,* and *Pantoea* were also Gram-negative rhizobacterial groups (Figure 4.6). The genus *Morganella* is the third dominant genus in this study. Accordingly, all of the strains under the genus *Morganella* had 99% similarity with *Morganella morganii* (Figure 4.6). The isolates BS-20 and RS-65 are grouped under *Serratia marcescens*. The remaining strains were classified under *Serratia grimesii* with 99% identity. Similarly, other genera of *Klebsiella* and

Pantoea had similarity indices with *Klebsiella michiganensis* and *Pantoea agglomerans*, respectively (Figure 4.6).

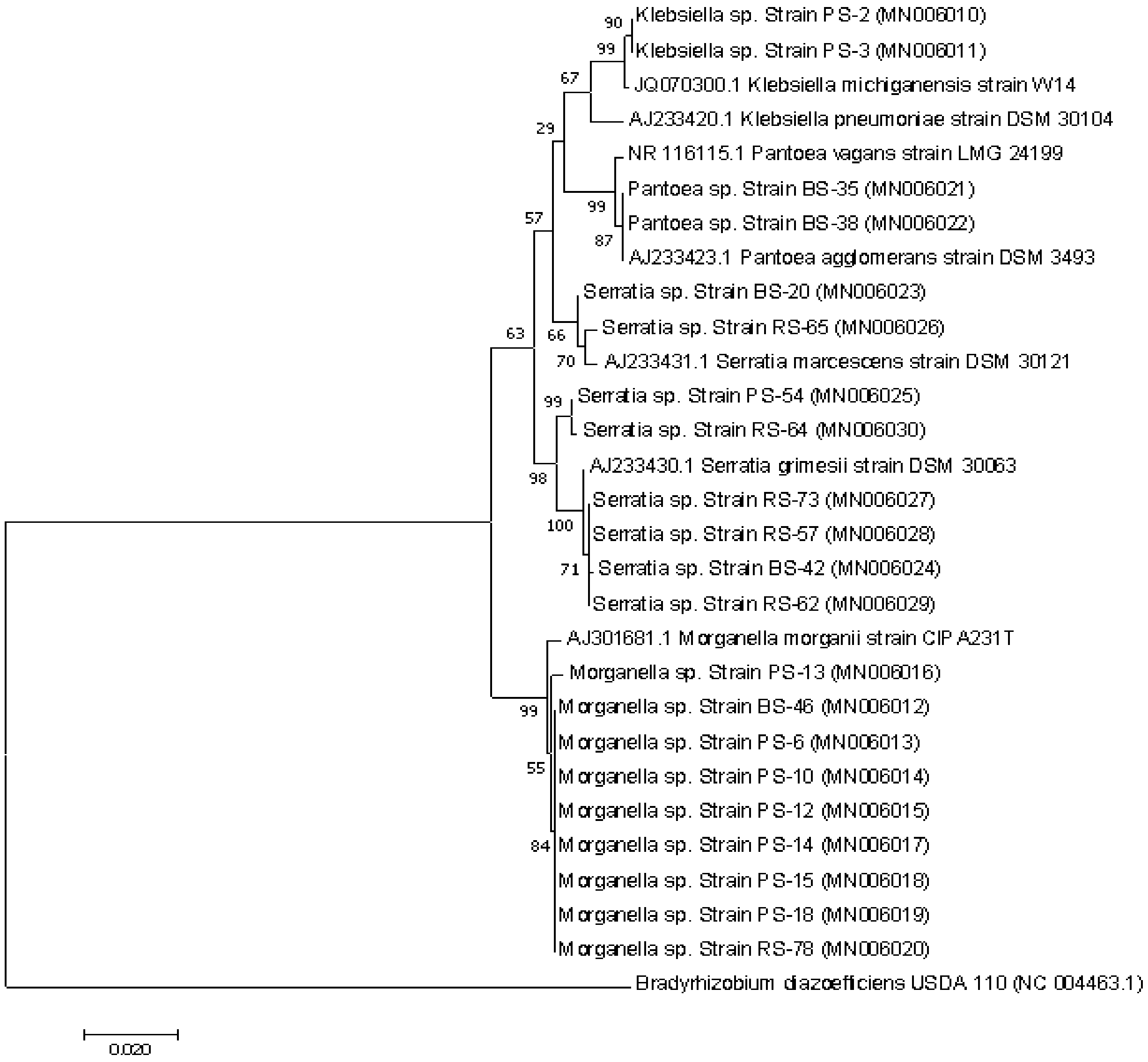

Figure 4.6. Phylogenetic tree of 16S rRNA gene sequences of Gram-negative rhizobacteria and some of their closest phylogenetic relatives using the Neighbor-Joining method.

The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. *Bradyrhizobium diazoeficiens* USDA 110 species was used for out grouping

The 16S rRNA gene sequence analysis showed the diversity of the 73 rhizobacteria isolates into different genera with 92-100% similarity indices (Table 4.5).

In general, the rhizobacterial isolates were diversified into 11 genera dominated by the genus *Pseudomonas* containing 17 isolates, followed by *Ochrobactrum* (15 isolates; Figure 4.7). But one isolate from *Pseudomonas* and two isolates from *Ochrobactrum* failed to get accession number and do not incorporated in the table. The genus *Morganella*, *Serratia*, *Bacillus,* and *Enterococcus* consisted of 9, 8, 7, and 6 isolates, respectively. (Figure 4.7, Table 4.5). These six genera constituted more than 85% of the population of the rhizobacteria recovered from degraded sampling sites (Table 4.5). Although the genus *Pseudomonas* was diversified into five species; *P. fulva, P. putida*, *P. protogens*, *P. fluorescens*, and *P. plecoglossicida*, the most dominant species was *P. fulva* that contained 75% of the population. The next dominant genus, *Ochrobactrum* also was diversified into *O. intermedium* and *O anthrop*i; where the former constitutes more than 90% of the population.

The genus *Morganella* was the third most widely distributed group represented by the only species; *M. morganii* which showed the same pattern as the genus *Enterococcus* that contained the only species; *Enterococcus gallinarum.* The Gm positive genera; *Bacillus* (*B. cereus*, *B. thuringensis,* and *B. anthracis*) and *Paenibacillus (P. odorifer* and *P. polymyxa)* were more diversified than the other dominant gm negative genera, except *Pseudomonas* and *Serratia,* and the minor group *Klebsiella*

Table 4.5. Summary table of the diversity of the isolates into their respective species and strains

Isolate	Closely related to	Accession description	% Gene identity	% Query coverage	No isolates
BS 22	*Bacillus anthracis*	CP033795.1	100	100	1
BS 29	*Bacillus cereus*	AY853168.1	100	99	1
BS 37	*Bacillus cereus*	AJ539175.1	99	99	1
BS 45	*Bacillus thuringensis*	KX641526.1	99	100	2
PS 4	*Enterococcus gallinarum*	CP033740.1	99	100	4
PS 11	*Enterococcus gallinarum*	JF915769.1	92	99	2
BS 51	*Paenibacillus polymyxa*	CP006872.1	100	100	1
BS 30	*Paenibacillus odorifer*	CP009281.1	100	100	1
FB 50	Paenibacillus polymyxa	CP025957.1	100	100	1
RS 71	Agrobacterium tumefaciens	CP033032.1	99	100	1
RS 58	*Ochrobactrum intermedium*	KC146415.1	100	99	4
RS 60	*Ochrobactrum intermedium*	AJ242582.2	99	99	8
RS 76	*Ochrobactrum antropi*	KC146415.2	100	100	1
BS 27	*Acinetobacter calcoaceticus*	KC257031.1	99	99	1
BS 19	*Pseudomonas putida*	CP025262.1	99	99	1
BS 21	*Pseudomonas fulva*	CP014025.1	100	99	12
BS 26	*Pseudomonas plecoglossicida*	MF281997.1	99	100	1
RS 75	*Pseudomonas protogens*	MK182884.1	99	100	1
FB 49	*Pseudomonas fluorescens*	KY228953.1	100	100	1
PS 1	*Klebsiella michiganensis*	CP033824.1	99	99	2
PS 3	*Klebsiella oxytoca*	CP033824.2	99	99	1
BS 46	*Morganella morganii*	CP032295.1	99	99	8
PS 13	*Morganella morganii*	HQ774675.1	99	100	1
BS 35	*Pantoea vagans*	CP014129.2	99	99	2
BS 20	*Serratia grimesii*	CP033162.1	99	100	4
BS 42	*Serratia grimesii*	MG972923.1	100	100	1
PS 54	*Serratia fonticola*	LR134492.1	99	100	2
RS 65	*Serratia marcescens*	CP021164.1	99	99	1

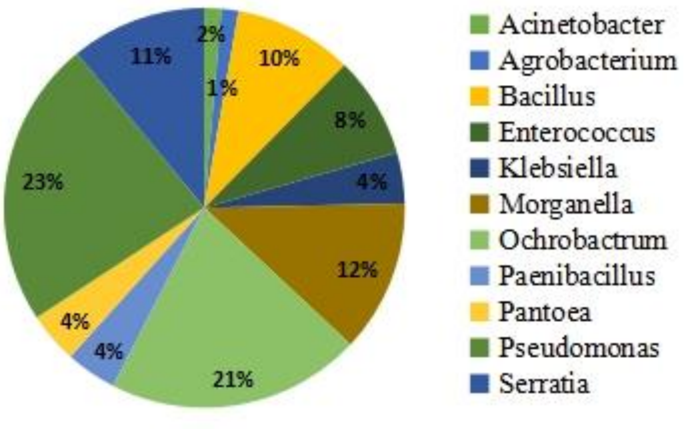

Figure 4.7. Distribution of rhizobacteria (genus level) identified by 16S rRNA genes sequencing. Values indicate percentages of strains belonging to each genus amongst the 73 identified strains.

4.4. Discussion

The rhizobacteria strain presents high metabolic diversities and can utilize all the carbohydrates (26.67 to 100%) and fewer amino acids (14. 28 to 100%) data not shown as it is long Table. This indicates that these rhizobacteria showed remarkable ecophysiological properties to utilize diverse biomolecules under a highly nutrient deficient environment. The ability to metabolize various carbon and amino acid sources is an indication that these isolates have numerous enzymes to hydrolyze available biomolecules as an energy source to survive under stressful habitats. This may play a significant role in the survival of the rhizobacteria to improve plant growth and yield even in hardy environments (Braga *et al.*, 2018).

The ability of rhizobacteria to utilize diverse organic substrates can be considered as an important trait for rhizosphere competence to make them a good candidate for the development of inoculants (Nannipieri *et al.*, 2003). Deng *et al.* (2011) reported that biomolecules exploitation can permit greater insight into the ecology and metabolism of microbial species and fundamentally essential in determining the functionality of that particular environment. Metabolic diversity profiling showed considerable diversity indices (Chojniak *et al.*, 2015). There is a plethora of information on microbial diversity in a vast range of environments (Escalas *et al.*, 2019).

In this study, the most differentiating DNA patterns for all rhizobacteria were obtained by using BOX - PCR that resulted in complex banding patterns, reflecting a high degree of genotypic diversity among them (Menna *et al.*, 2009). The taxonomic data showed that BOX-PCR polymorphism patterns have been effectively used for the differentiation of bacterial strains (Louws *et al.*, 1994).

In this study, the highest diversity index was recorded from *Bacillus* species that may indicate their ability to form resistant spores to adapt to that particular degraded environment. Simpson's Index gives more weight to the more abundant species in a sample. A similar result of Simpson’s Index of Diversity (D) of BOX-PCR (0.888) was reported for *Listeria* spp. and *Listeria monocytogenes* (Maurice Bilung *et al.*, 2018). Moreover, the genotypic diversity in *Bacillus* spp was reported using BOX PCR patterns (Köberl *et al.*, 2011).

In this study, two major phyla and 11 genera of rhizobacteria were identified with 92-100% similarity indices and confirmed by lower E-values. The genera *Pseudomonas* and *Ochrobactrum* were the dominant groups in the phylum Proteobacteria was dominated herby contributing where the two genera 44% of the total population of the rhizobacteria. On the other hand, Firmicutes constituted the genera *Bacillus* and *Enterococcus*. Some Gram-positive genera of *Bacillus, Enterococcus,* and *Paenibacillus* were characterized. The dominance of Proteobacteria is of great importance to global carbon, nitrogen, and sulfur cycling to ensure sustainable biogeochemical cycling processes (Itävaara *et al.,* 2016). The authors reported that Proteobacteria constitutes the largest and phenotypically most diverse and considered a dominant microbial clade.

Similar to this finding, Proteobacteria (25.10%) and Firmicutes (24.8%) were reported as the most abundant from the Taklamakan desert, in Asia (China) (An *et al.*, 2013). In another Asian desert, the Gobi Desert, confirming the dominance of Firmicutes (69.9 %) and Proteobacteria (12.2%) phyla (An *et al.*, 2013). In the dry soil, *Ochrobactrum* sp. was the most abundant (79%) genus while *Bacillus* and *Paenibacillus* were found (5%) from some medicinal plants (Köberl *et al.*, 2011).

In contrast, in pine forest soil, 29.41% and 35.29% Proteobacteria and Firmicutes phyla were distributed (Flores-Núñez *et al.*, 2018). *Pseudomonas* (six species) and *Bacillus* (four species) were identified from wild *Coffea arabica* while *Ochrobactrum* and *Serratia* were also identified as single species (Diriba Muleta *et al.*, 2009). Another study indicated bacterial diversity isolated from kauri (*Agathis australis*) forest with exotic pine (*Pinus radiata*) plantation forest showed Simpson's diversity index of 0.98 (Byers *et al.*, 2020).

A higher genetic divergence was evident in the *O. intermedium* than that of *O. anthropic*. Based on phenotypic characteristics, the genus *Ochrobactrum* could be related to the genera *Alcaligenes, Achromobacter*, or the members of *Pseudomonadaceae*. However, molecular taxonomy places *Ochrobactrum* in the α-subgroup of proteobacteria that closely related to the genus *Brucella* (Velasco *et al.*, 1998). Surprisingly, 16S rDNA-based phylogeny as well as protein profiling Velasco *et al.* (1998) and AFLP analysis Leal-Klevezas *et al.* (2005) placed *O. intermedium* strains closer to *Brucella* spp. than any other members of the genus *Ochrobactrum*.

Even though there is no generally accepted cut-off value for the bacterial species delineation, a 97% similarity level in 16S rDNA has been proposed (Stackebrandt and Geobel, 1994). According to this value, *O. anthropic* and *O. intermedium* were not separated. Although *Ochrobactrum intermedium* is currently reported as opportunistic pathogens in humans Teyssier *et al.* (2005), there are some reports on the presence of *Ochrobactrum* spp. from different environments including soil. Huber *et al.* (2010), the rhizosphere and in internal root tissues of different plants (Trujillo *et al.*, 2005). Some nodulating species of *Ochrobactrum* spp. have been described that form nodules on *Acacia* Ngom *et al.* (2004) and *Lupinus* (Trujillo *et al.*, 2005). *O. intermedium* increased seed germination, root and shoot length, and grain yield in lentil (*Lens esculenta*) (Faisal, 2013). The first plant promoting roles of *O. intermedium* was reported as it increases the peanut shoot and root height as well as dry weight (Paulucci *et al.*, 2015). Moreover, in vitro studies confirmed that *Ochrobactrum* spp. and others were the most important isolates to act as potential biofertilizers, biocontrol agents, or both (Diriba Muleta, 2007).

In this study, some strains of *Morganella* were recovered from degraded soil. A previous study showed that an endophytic *M. morganii* was reported to be effective when applied to the seeds with significantly higher plant growth promotion than the control (Shiomi, 2007). This may be associated with the gene encoding for acid phosphatases. In earlier investigations, several acid phosphatase genes have been isolated and characterized from Gram-negative bacteria (Rossolini *et al.*, 1998). Similarly, the *acpA* gene isolated from *Francisella tularensis* expressed an acid phosphatase with optimum action at pH 6 with a wide range of substrate specificity (Reilly *et al.*, 1996). Similarly, the *napA* phosphatase gene from the soil bacterium *M. morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as a biofertilizer using the broad-host-range vector pRK293 (Fraga *et al.*, 2001). Generally, the current study showed degraded soil could harbor metabolically and genetically diverse rhizobacteria. This could help to adapt to harsh environments and be involved in plant growth-promoting activities is an implication for potential source for inocula development.

4.5. Conclusion

Degraded soil harbored metabolically diverse rhizobacterial genera of *Ochrobactrum* and *Pseudomonas* taking the leading position. BOX-PCR showed a better discriminatory power and differentiating DNA patterns for all strains and revealed high genotypic diversity. Based on the genotyping analysis, PGPR isolates were heterogeneous with a high index of genetic diversity.

Chapter 5

5. Taxonomic and Functional Diversity of Plant Growth Promoting Rhizobacteria to Enhance the Growth of Seedlings of Acacia (*Acacia abyssinica* Hochst. ex Benth) on Soils Collected from Degraded Habitat under Greenhouse Conditions

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Abstract

Drought stress (DS) is one of the most important factors that affect the ecological balance of a particular habitat. This is particularly severe in degraded soil where plant and microbial diversity have been lost which makes restoration very difficult. Plant growth-promoting rhizobacteria (PGPR) are one of the most important groups necessary to support the restoration of plants in a certain habitat. The search for potential PGPR capable of enhancing plant tolerance to drought stress is all the more important to rehabilitate degraded lands with plants. This study evaluated the effect of inoculating *Acacia abyssinica* seedlings with PGPR isolated from degraded sandy clay loam soil of Ethiopia, aiming at their use to enhance DS tolerance. The strains were selected based on *in vitro* assays associated with tolerance to drought and other beneficial traits such as salinity, acidity, temperature, heavy metal tolerances, and biofilm formation. The strains with the best DS tolerance ability were selected for the greenhouse trials with acacia plants. The results indicate that out of 73 isolates, 10 (14%) were highly tolerant of 40% polyethylene glycol. Moreover, 37% of the isolates were strong biofilm producers. Isolates PS-16 and RS-79 showed tolerance to 11% NaCl. All the strains were able to grow in wider ranges of pH (4-10), temperature (15-45°C), and showed variations in resistance to the tested heavy metals ranging between 50% and 100%. The inoculated bacterial isolates significantly ($p \leq 0.05$) increased root and shoot length and dry biomass of acacia plants. Inoculation with bacterial consortia had significantly ($p \leq 0.05$) enhanced plant biomass 57.3 cm, 19.3 cm, 2.1 g, 0.8 g, and 16.7 in SH, RL, SDW, and RDW and number of leaves per plant, respectively compared to non-inoculated control. The inoculation of the different rhizobacteria showed a significant difference in shoot dry weight (SDW) ranging from 1.5 g/pot to 2.9 g/pot (mean 1.93 g/pot) under normal conditions. Under stress conditions, the shoot dry matter yield was within the range of 1.1 g/pot-1.8 g/pot (mean 1.44 g/pot) with an overall reduction of 25%. One of the isolates identified as *P. fluorescens* was outstanding in enhancing DS tolerance compared to the single inoculants and comparable to consortia. Stress tolerant PGPR could be used to enhance Acacia DS tolerance after testing other beneficial traits.

Keywords: Beneficial microbes, Fabaceae, Greenhouse, Inoculation, Stresses alleviations

5.1. Introduction

Land degradation severely affects the ecological stability of a given habitat Glick (2014), and increases episodes of drought, salinity, toxicity by heavy metals, soil acidity (Ma *et al.*, 2019). Drought stress (DS) is the most impacting phenomena that affect ecological integrity caused by loss of plant and soil microbial diversity, soil fertility and ultimately result in poor agricultural productivity (Khan *et al.*, 2019). Soil bacteria inhabiting salty and arid ecosystems have the potential to promote plant growth under salinity and drought conditions (Mapelli *et al.*, 2013). Successful deployment of PGPR in stressed ecosystems depends on their ability to withstand and proliferate under adverse environments (Gopalakrishnan *et al.*, 2015).

Plants have evolved different mechanisms to mitigate DS, including a series of molecular, cellular, and physiological adaptations (Saleem *et al.*, 2018). Chodak *et al.* (2015) call for urgent intervention measures using innovative and eco-friendly approaches. Hence, integrating drought-tolerant beneficial microbes as a component of ecological systems to enhance plant drought tolerance might represent an interesting strategy to improve environmental integrity and stability. Bashan *et al.* (2014) described that the interactions between microbes and plants for enhancing plant stress resistance stimulated great interests among researchers. At the moment, efforts have been focused on harnessing the potential of phytobeneficial soil microbes to enhance crop production and environmental rehabilitation to combat the negative impacts of drought (Kaushal, 2019). The positive influence of PGPR on conferring resistance to DS in many crops and trees have been reported (Bashan *et al.*, 2014).

Microorganisms also confer tolerance and survival by different inherent characters that include the formation of microbial biofilm and exopolysaccharides for protection from external stress and to decrease microbial competition and increase soil aggregation (Asari, 2015). Sandhya *et al.* (2010) have been documented the remarkable potential of many PGPR genera including *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Paenibacillus* and *Bacillus* in enhancing plant stress tolerance in dryland areas. Plant growth promoting rhizobacteria can enhance plant stress tolerance by an array of mechanisms that encompass the production of ACC deaminase Gou *et al.* (2015), regulation of the hormonal balance of cytokinins (Shilev, 2013). Screening for stress tolerance is an important parameter while selecting bacterial strains for the development of biofertilizers since the performance of PGPR is constrained by environmental stresses including temperature, desiccation, pH, alkalinity/acidity and salinity in the soil (Das *et al.*, 2015). Due to their multiple traits, the search for suitable and rhizosphere competent PGPR becomes interesting, once they can be used as inoculants for bio-fertilization, phytostimulation, and bio-control purposes in agriculture, forestry, and environmental rehabilitation.

Acacia is highly used for conserving and improving degraded soils and landscapes. Therefore, the selection of stress-tolerant bacterial strains might be critical for improving field performance of potent microbial strains. Hence, this work aimed to identify and characterize PGPR isolates that could enhance stress tolerance by promoting the growth of Acacia seedlings.

5.2. Materials and Methods

5.2.1. Rhizobacteria Growth Conditions and Identification

Rhizobacterial isolates were isolated from the woody plants described above in Chapter 4 and deposited at culture collection of the Addis Ababa University at the Department of Microbial Cellular and Molecular (MCMB) that had been previously collected, properly identified (genetic), and deposited in gene banks elsewhere (Table 5.1) (Alemayehu Getahun *et al.*, 2020c).

Table 5.1. Plant-growth promoting rhizobacteria (PGPR) isolates tested for their drought stress (DS) capacity under in vitro conditions. Their closest species identity based on the 16S rRNA gene sequencing analysis

Isolate code	The closest relatives	Best match ID (NCBI)	Query cover (%)	% Similarity	Gene bank accessions
BS-45	*Bacillus* thuringiensis	CP021436.1	100	100	MN005965
PS-16	*Enterococcus gallinarum*	JF915769.1	99	99	MN005973
FB-50	*Paenibacillus polymyxa*	CP025957.1	100	100	MN005976
RS-79	*Agrobacterium tumefaciens*	CP033032.1	100	99	MN005977
RS-72	*Ochrobactrum intermedium*	KC146415.1	100	100	MN005988
BS-27	*Acinetobacter calcoaceticus*	KC257031.1	99	99	MN005992
BS-19	*Pseudomonas putida*	CP025262.1	99	99	MN005993
FB-49	*Pseudomonas fluorescens*	KY228953.1	100	100	MN006008
PS-6	*Morganella morganii*	CP032295.1	100	99	MN006013
RS-65	*Serratia marcescens*	CP021164.1	99	99	MN006026

5.2.2. Screening Drought Stress (DS) Tolerant of PGPR

The rhizobacteria were screened for potential tolerance to drought (DS) using polyethylene glycol (PEG) and for their plant growth-promoting properties using standard methods. The twenty-two genetically identified isolates were tested for *in vitro* plant growth promoting traits and drought tolerance (osmotic stress) following the method of Alikhani and Mohamadi (2010). Thus, 1 mL of the bacterial culture at the concentration of $1x10^7$/mL was inoculated into Tryptic Soya Broth (TSB) g/L: pancreatic digest of casein 17; a peptic digest of soya bean meal 3; sodium chloride 5; dextrose 2.5 and dibasic potassium phosphate 2.5; 40% of polyethylene glycol- to adjust the osmotic pressure at 1.76 MPa into the medium and incubated, at 28±2°C for 24 h and OD was recorded after 3 days. The OD values of drought tolerance were determined as: Highly Sensitive OD < 0.3; Sensitive OD = (0.3-0.39); Tolerant OD = (0.4-0.5) and Highly Tolerant OD > 0.5.

5.2.3. Qualitative and quantitative assay for biofilm formation

5.2.3.1. Plate Method (PM)

The ability to produce biofilm was tested using the method of Mathur *et al.* (2006). Thus, eighteen hours old bacterial cultures were streaked on the Congo Red Agar (CRA) plates containing (g/L): brain heart infusion broth, 37; sucrose, 5, agar, 10; Congo red dye, 0.8 and incubated at 28 ±2°C for 24-48 h. Black colonies with a dry crystalline consistency indicate biofilm production.

5.2.3.2. Tube Method (TM)

Biofilm formation ability was observed by its adherence capacity to the walls of culture tubes (Mathur *et al.*, 2006). A loop-full of each bacterial strain grown on TSB plates for 24 h was inoculated into 10 mL of nutrient broth with different NaCl concentrations (100 and 150 mM which was used to show enhanced absorbance) in test tubes followed by shaking at 95 rpm for 24-48 h. The culture medium with bacteria was discarded and the tubes were washed with 3 mL of 1X phosphate buffer saline (PBS) of pH 7. A 3 mL of 2% crystal violet solution was added and left for

15 min. Tubes were then washed with sterile water, allowed to dry and the tubes were visually observed for the presence of biofilms rings on the inner walls of the test tubes. Tubes then received 1.5 mL of 33% glacial acetic acid and mixed gently to measure OD at 570 nm. Phosphate buffer saline served as control. Biofilm formation in tubes was detected when a visible film (ring) lined the wall and the bottom of the tubes.

5.2.4. Growth and eco-Physiological Characterization

For each biochemical and physiological test, growth was determined by reading OD at 600 nm in Nutrient Broth (g/L): peptone, 5; NaCl, 5; beef extract, 1.5; yeast extract, 1.5. In all cases of tolerance measurements, the viability of the isolates was checked by streaking on TSA immediately following the OD reading and incubated at 28 ± 2°C for 3-5 days (Solomon Legesse and Fassil Assefa, 2014).

5.2.4.1. Salt, pH, Temperature, and Heavy metal tolerance tests

Tolerance to salinity was evaluated by growth on TSA medium containing 1%, 3%, 5%, 7%, 9% and 11% (w/v) NaCl (Romdhane *et al.*, 2009). pH tolerance was tested in nutrient broth by adjusting the pH to 4, 5, 6, 8, and 10 with either 1N NaOH or HCl (Küçük *et al.*, 2006). Temperature tolerance was evaluated by growing bacterial cultures in TSA at 4°C, 15°C, 25°C, 35°C, 40°C, 45°C and 50°C. The agar dilution method was used to test the heavy metal (HM) tolerance of PGPR isolates (Lee *et al.*, 2009). A loop-full of 24 h-old bacterial culture grown in the nutrient broth was streaked on

Muller-Hinton Agar (MHA) Messi *et al.* (2005) plate amended with increasing concentrations (50, 100 and 300 µg/mL) of different heavy metals lead from $(PbCH_3COO)_2.3H_2O$, zinc from $ZnSO_4.5H_2O$, copper from $CuCl_2.2H_2O$, manganese from $MnSO_4.4H_2O$ and iron from $FeSO_4.6H_2O$). Plates were incubated, at 28 ±2°C for 24 h and examined visually for the presence or absence of growth where the presence of growth was recorded as resistance/tolerance (R), whereas the absence of growth was recorded as susceptible used as controls to evaluate tolerance (Hung *et al.*, 2005).

5.2.5. Greenhouse Experimental Trails

Pot trials were performed under greenhouse conditions at the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University to evaluate the potential of the selected PGPR on the growth of *Acacia abyssinica* plants. *A. abyssinica* seeds were collected from different locations of the highland region of Ethiopia and were scarified with concentrated H_2SO_4 in flasks to break seed dormancy. Eight seeds were kept in equidistance position in sterilized Petri plates containing sterile moist filter paper and cotton for 7 days for germination (Mia *et al.*, 2012). After germination, four seedlings were transplanted into sterile plastic pots (20 × 15 cm) filled with 3 kg sandy clay loam soil autoclaved for 1h. Plants were kept in well-watered conditions and fertilized with half-strength Hoagland solution each week to obtain nutrients at a free access rate for 30 days (Saleem *et al.*, 2018). Plants were inoculated during and 7 days post-transplantation with test strains (10^8 CFU/mL) for 60 days. Drought stress was induced by stopping watering after 30 days of growth. The experiment was performed with a completely randomized design and replicated three times. Plants were harvested after 2 weeks of water stress and data on root length, shoot length, root and shoot dry biomass were recorded.

5.2.6. Data Analysis

Analysis of Variance (ANOVA) was used to test for significant differences of measurements of each bioassay, whereas Duncan's Multiple Range Test (DMRT) was employed to show the significant difference among diverse treatments (mean separation) at $p \leq 0.05$. Values are presented as means ± standard deviation (SD). All the statistical analyses were performed using Statistical Analysis System (SAS) version 9.0 software package. Phylogenetic analysis of partial 16S rRNA gene sequences was done using Mega 7 software version 7.0.2. (Kumar *et al.*, 2016).

5.3. Results

5.3.1. Tolerance of rhizobacteria to stresses

5.3.1.1. Drought Stress

The ten PGPR isolates were selected from the 73 isolates based on their DS tolerance. Bacterial isolates showed high drought tolerance (HT) (Table 5.2). *Pseudomonas fluorescens* FB-49 showed the highest 0.64 OD value followed by *Pseudomonas putida* BS-19 with 0.60 OD.

Table 5.2. In vitro features of the strains selected for Acacia abyssinica growth promotion under water stress n=3.

S. No.	Isolate code	The closest relatives	Mean ±SD 600 nm OD	Tolerance Levels
1	BS-45	*Bacillus thuringiensis*	0.56±0.12	HT
2	PS-16	*Enterococcus gallinarum*	0.54±0.17	HT
3	FB-50	*Paenibacillus polymyxa*	0.51±0.16	HT
4	RS-79	*Agrobacterium tumefaciens*	0.57±0.20	HT
5	RS-72	*Ochrobactrum intermedium*	0.52±0.22	HT
6	BS-27	*Acinetobacter calcoaceticus*	0.59±0.19	HT
7	BS-19	*Pseudomonas putida*	0.60±0.12	HT
8	FB-49	*Pseudomonas fluorescens*	0.64±0.15	HT
9	PS-2	*Klebsiella michiganensis*	0.50±0.17	HT
10	RS-65	*Serratia marcescens*	0.55±0.15	HT

5.3.2. Salt, pH, Temperature, and Heavy metal tolerance

5.3.2.1. Tolerance to different NaCl Concentrations

The data showed that the PGPR strains could grow over a wide range of NaCl (1 to 11%) concentrations (Table 5.3). Thus, *Enterococcus gallinarum* PS-16 and *Agrobacterium tumefaciens* RS-79 showed the highest NaCl tolerance followed by *Pseudomonas fluorescens* FB-49 and *Paenibacillus polymyxa* FB-50 with 9%. *Pseudomonas putida* BS-19 was the most sensitive to salt concentration. However, higher NaCl concentration led to a drastic reduction in the growth of bacterial isolates.

Table 5.3. The effect of salt tolerance of the rhizobacteria grown on TSA medium at 28°C for 3-5 days (measured based on OD reading

Species	**NaCl concentration (OD)**					
	1%	3%	5%	7%	9%	11%
A. calcoaceticus BS-27	1.02±0.16^{c}	0.84±0.23^{e}	0.77±0.21^{ce}	0.70±0.28^{cde}	0.06±0.02^{e}	0.02±0.03^{b}
P. polymyxa PS-50	1.66±0.29^{a}	1.23±0.08^{bc}	1.08±0.03^{bc}	0.96±0.12^{abc}	0.52±0.12^{d}	0.08±0.08^{b}
B. thuringiensis BS-45	1.52±0.25^{ab}	1.22±0.05^{bc}	1.06±0.16^{ab}	0.88±0.13^{bcd}	0.49±0.05^{cd}	0.05±0.03^{b}
P. fluorescens FB-49	1.77±0.47^{a}	1.43±0.32^{ab}	1.31±0.10^{a}	1.02±0.09^{ab}	0.61±0.17^{bc}	0.08±0.02^{b}
O. intermedium RS-72	1.20±0.10^{bc}	1.10±0.09^{cd}	0.91±0.10^{a}	0.63±0.07^{de}	0.44±0.05^{d}	0.08±0.03^{b}
P. putida BS-19	1.00±0.10^{c}	0.95±0.13^{e}	0.86±0.06^{bcd}	0.82±0.07^{bcde}	0.50±0.15^{d}	0.05±0.03^{b}
A. tumefaciens RS -79	1.89±0.11^{a}	1.52±0.07^{a}	1.10±0.2^{bc}	1.04±0.14^{ab}	0.73±0.04^{b}	0.07±0.04^{b}
E. gallinarum PS-16	1.85±0.21^{a}	1.49±0.07^{a}	1.16±0.02^{ab}	1.17±0.04^{a}	0.88±0.08^{a}	0.32±0.06^{a}
S. marcescens RS-65	1.20±0.1^{bc}	0.95±0.07^{de}	0.70±0.15^{e}	0.56±0.13^{e}	0.38±0.03^{d}	0.05±0.04^{b}
K. michiganensis PS-2	1.27±0.07^{bc}	1.04±0.05^{cde}	0.76±0.17^{de}	0.66±0.21^{de}	0.17±0.05^{e}	0.07±0.03^{b}

Values represent the mean ± SD of three replicates. Treatments followed by different letters indicate significant difference over control using Duncan's multiple range test ($p \leq 0.05$) n=3

5.3.2.2. Growth at different pH ranges

The data also showed that the PGPR were tolerant to a wide pH range suggesting that they can survive in both acidic and alkaline environments. All isolates showed maximum growth at pH 7 followed by pH 10. However, fewer isolates were tolerant to pH 5. Thus, *Agrobacterium tumefaciens* RS-79, *Pseudomonas fluorescens* FB-49, *Serratia marcescens* BS-65, and *Klebsiella michiganensis* PS-2 grew better at pH 5; whereas the most resistant species *A. tumefaciens* RS-79, *P. fluorescens* FB-49 were also able to grow at pH 4 data not shown (Table 5.4).

Table 5.4. Growth determined at OD 600 nm of selected PGPR isolates at varying pH ranges

Species	pH5	pH7	pH10
Acinetobacter calcoaceticus BS-27	0.44 ± 0.04^{cd}	1.26 ± 0.21^{abcd}	1.13 ± 0.09^{abc}
Paenibacillus polymyxa PS-50	0.43 ± 0.05^{cd}	1.32 ± 0.19^{abcd}	1.11 ± 0.12^{abc}
Bacillus thuringiensis BS-45	0.15 ± 0.05^{e}	1.39 ± 0.09^{ab}	1.17 ± 0.11^{ab}
Pseudomonas fluorescens FB-49	0.67 ± 0.09^{bc}	1.46 ± 0.11^{a}	1.32 ± 0.23^{a}
Ochrobactrum intermedium RS-72	0.24 ± 0.05^{de}	1.05 ± 0.04^{b}	0.97 ± 0.07^{bcd}
Pseudomonas putida BS-19	0.14 ± 0.06^{e}	1.11 ± 0.10^{bcd}	0.80 ± 0.11^{cd}
Agrobacterium tumefaciens RS -79	0.84 ± 0.06^{a}	1.20 ± 0.14^{abcd}	0.70 ± 0.15^{d}
Enterococcus gallinarum PS-16	0.42 ± 0.05^{cd}	1.39 ± 0.06^{ab}	1.13 ± 0.116^{abc}
Serratia marcescens RS-65	0.63 ± 0.05^{b}	1.09 ± 0.10^{cd}	$1.01\pm0.04a^{bcd}$
Klebsiella michiganensis PS-2	0.49 ± 0.08^{bc}	1.34 ± 0.03^{abc}	1.18 ± 0.03^{ab}

Means with the same letter down the column are not significantly different. Mean ± SD of three replicates using Duncan's multiple range test ($p \leq 0.05$) n=3

5.3.2.3. Response to different Temperatures

All isolates were able to grow within a broad range of temperature (20°C to 45°C), but failed to grow at 4°C and 50°C. Maximum growth was achieved at 25°C, 35°C and 40°C, but lower at 15°C and 45°C (Table 5.5). Five (FB-50, RS-45, FB-49, and RS-79) isolates exhibited remarkable tolerance to high temperature (45°C) followed by BS-19.

Table 5.5. Effect of temperature on the growth of selected PGPR isolates OD readings at 600 nm

Isolates	Temp 15°C	Temp 25°C	Temp 35°C	Temp 40°C	Temp 45°C
BS-27	0.30 ± 0.04^{a}	0.89 ± 0.07^{ab}	1.13 ± 0.04^{abc}	0.89 ± 0.14^{abc}	0.55 ± 0.05^{b}
FB-50	0.25 ± 0.07^{abc}	0.98 ± 0.11^{ab}	1.16 ± 0.02^{abc}	0.82 ± 0.15^{bc}	1.01 ± 0.09^{a}
RS-45	0.19 ± 0.04^{abc}	0.85 ± 0.17^{ab}	0.87 ± 0.05^{bc}	1.12 ± 0.02^{abc}	0.95 ± 0.11^{a}
FB-49	0.26 ± 0.05^{ab}	1.21 ± 0.14^{a}	1.36 ± 0.19^{ab}	1.10 ± 0.12^{a}	0.94 ± 0.14^{a}
RS-72	0.16 ± 0.01^{bc}	0.76 ± 0.05^{b}	1.05 ± 0.02^{c}	0.73 ± 0.03^{c}	0.29 ± 0.03^{b}
BS-19	0.22 ± 0.02^{abc}	0.93 ± 0.07^{ab}	1.39 ± 0.09^{a}	0.85 ± 0.07^{abc}	0.53 ± 0.05^{b}
RS-79	0.21 ± 0.04^{abc}	0.80 ± 0.13^{b}	1.29 ± 0.07^{abc}	0.73 ± 0.05^{c}	0.55 ± 0.03^{b}
PS-16	0.17 ± 0.02^{abc}	0.98 ± 0.09^{ab}	1.13 ± 0.07^{abc}	0.92 ± 0.10^{abc}	0.45 ± 0.07^{b}
BS-65	0.21 ± 0.03^{abc}	0.90 ± 0.11^{ab}	1.15 ± 0.09^{abc}	0.72 ± 0.09^{c}	0.52 ± 0.04^{b}
PS-2	0.12 ± 0.02^{c}	0.75 ± 0.06^{b}	1.14 ± 0.07^{abc}	1.07 ± 0.06^{ab}	0.47 ± 0.09^{b}

Means with the same letter down the column are not significantly different. Mean ± SD of three replicates using Duncan's multiple range test ($p \leq 0.05$) n=3

5.3.2.4. Tolerance of PGPR to heavy metals (HMs)

Many isolates were tolerant to various concentrations of heavy metals tested (Figure 5.1). All isolates (100%) showed resistance to 50 µg/mL of, Cu, Zn and Pb, whereas almost all isolates were able to grow on the medium containing 100 µg/mL of Fe, Mn. However, fewer isolates grew on the medium containing the same concentration of Zn (77% of the isolates, Pb (73%) and Cu (67%), respectively. Moreover, bacteria growth significantly declined ($p \leq 0.05$) to 32% - 44 at 300 µg/mL increase in concentrations of the heavy metals, except Fe (Table 5.6).

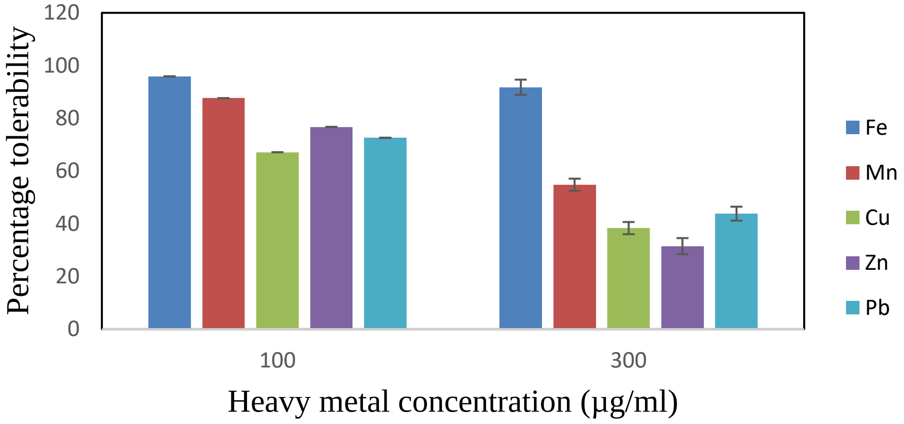

Figure 5.1. Tolerance of PGPR isolates grown on Muller-Hinton Agar at 28 ±2°C for 24 h to HM concentration.

The different letters on the standard error (SD) bars indicate a significant difference using Duncan multiple range test at $p \leq 0.05$. Values are means of three replicates

The inter-species difference in resistance of different heavy metals among the rhizobacteria is shown on Table 5.6. The data showed that the different species showed variations in resistance to the tested heavy metals ranging between 50% and 100%. Thus, all the pseudomonas spp (*Pseudomonas putida* BS-19, *Pseudomonas fluorescens* FB-49, *Pseudomonas fluorescens* FB-49), *Paenibacillus polymyxa* FB-50, and *Acinetobacter calcoaceticus* BS-27 were tolerant to all tested heavy metals and concentrations. The sensitive strains were *Ochrobactrum intermedium* RS-72 and *Serratia marcescens* RS-65 that were able to grow on 50% of the tests.

Table 5.6. Heavy metal tolerance profile of ten potential PGPR strains at varying concentrations of HMs. T = Tolerance and S = Sensitive

		Heavy Metals (µg/ml)															
S. No.	Isolates	Fe 50	Fe 100	Fe 300	Mn 50	Mn 100	Mn 300	Cu 50	Cu 100	Cu 300	Zn 50	Zn 100	Zn 300	Pb 50	Pb 100	Pb 300	**% (R)**
1	*Bacillus thuringiensis* BS-45	T	T	T	T	T	S	T	T	T	T	T	T	T	T	T	**90**
2	*Enterococcus gallinarum* PS-16	T	T	T	T	T	T	T	S	S	T	T	S	T	T	T	**70**
3	*Paenibacillus polymyxa* FB-50	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	**100**
4	*Agrobacterium tumefaciens* RS-79	T	T	T	T	T	T	T	T	S	T	T	S	T	T	T	**80**
5	*Ochrobactrum intermedium* RS-72	T	T	T	T	T	S	T	T	T	T	S	S	T	S	S	**50**
6	*Acinetobacter calcoaceticus* BS-27	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	**100**
7	*Pseudomonas putida* BS-19	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	**100**
8	*Pseudomonas fluorescens* FB-49	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	**100**
9	*Klebsiella michiganensis* PS-2	T	T	T	T	T	S	T	T	T	T	S	S	T	T	T	**70**
10	*Serratia marcescens* RS-65	T	T	T	T	T	S	T	S	S	T	S	S	T	T	T	**50**
	% (T)	**100**	**100**	**100**	**100**	**100**	**70**	**100**	**80**	**70**	**100**	**70**	**60**	**100**	**90**	**90**	
	% (S)	**0**	**0**	**0**	**0**	**0**	**30**	**0**	**20**	**30**	**0**	**30**	**40**	**0**	**10**	**10**	

5.3.3. Qualitative Biofilm Detection

Most of the isolates produced black colored colonies on CRA after 24-48 h. In the tube method, the formation of visible thick film inside the wall of tubes and their bottom. Out of 73, 27 (37%) isolates were strong, 49% moderate biofilm producers, and the remaining (14%) were leveled as weak or non-biofilm producers using TM at 150 mM NaCl. By using TM but with different NaCl concentrations (100 mM), 10.12%, 55.16%, and 34.72% were perceived as strong, moderate, and weak biofilm-producing PGPR isolates, respectively (Figure 5.2).

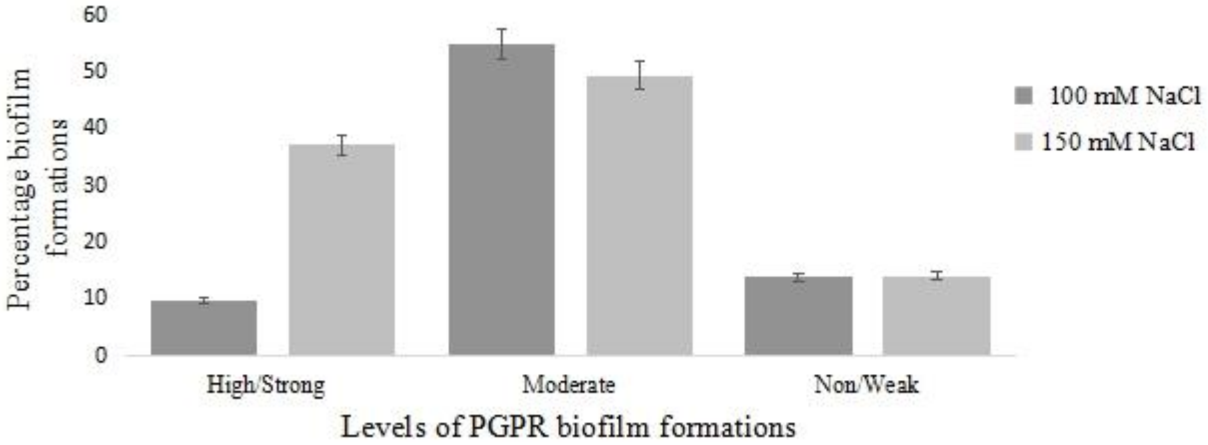

Figure 5.2. Classification and comparisons of bacterial biofilm formation abilities at 100 mM and NaCl 150 mM NaCl concentrations

The results indicated that the activity of biofilm formation was increased with increasing NaCl concentration. The highest significant increase was recorded in *Pseudomonas putida* BS-19 and *Pseudomonas fluorescent* FB-49 with 0.805 and 0.765 OD respectively at 150 mM NaCl while the lowest was observed in *Klebsiella michiganensis* PS-2 0.39 OD (Figure 5.3).

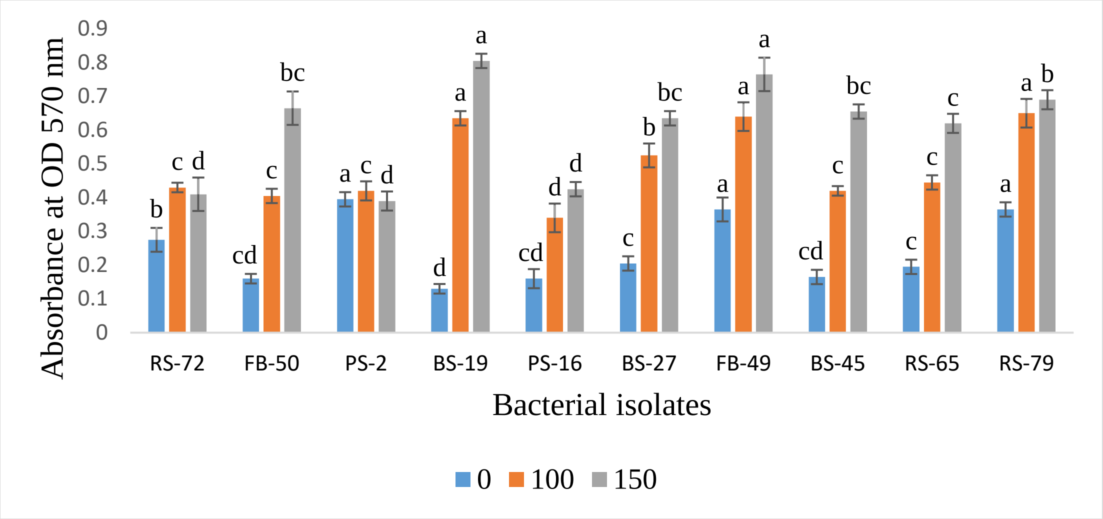

Figure 5.3. The optical density (OD at 570 nm) as the measure of the activity of biofilm formation for PGPR isolates with three NaCl (0, 100, and 150 mM) concentrations

5.3.4. Drought Stress Enhancements in Acacia under Greenhouse

Inoculation with bacterial consortia had significantly ($p \leq 0.05$) enhanced plant biomass 57.3 cm, 19.3 cm, 2.1 g, 0.8 g, and 16.7 in SH, RL, SDW, and RDW and number of leaves per plant, respectively compared to non-inoculated control (Table 5.7). Of a single inoculation *Pseudomonas fluorescens* FB-49 significantly enhanced the plant biomass 51.7 cm, 18.7 cm, 1.8 g, 0.55 g, and 16.6 in SH, RL, SDW, and RDW and number of leaves per plant, respectively compared to non-inoculated control (Table 5.7).

The inoculation of the different rhizobacteria showed a significant difference in shoot dry weight (SDW) ranging from 1.5 g/pot to 2.9 g/pot (mean 1.93 g/pot) under normal conditions (Table 5.7). Under stress conditions, the shoot dry matter yield was within the range of 1.1 g/pot-1.8 g/pot (mean 1.44 g/pot) with an overall reduction of 25%. The inoculated plants produced higher biomass i.e., 40-70% than the uninoculated plants. The highest dry weight was obtained from consortia inoculation of the isolates, (2.9 g/pot), followed by single inoculation with *Bacillus thuringensis* BS-

45 with shoot dry biomass yield of 2.4 g/pot, followed by *Pseudomonas putida* BS-19 and *Pseudomonas fluorescens* FB-49, with shoot dry mass accumulation of 2.2 g/pot and *Ochrobactrum* RS-72 with 2.1 g/pot, respectively

The inoculants showed variations in their effect on drought stress regarding the shoot dry weight of the host plant (Table 5.7). In all cases shoot dry weight significantly decreased (11%-42%) with an overall mean decrease of 25% compared to the normal treatment as a result of drought stress. The uninoculated plants with and without drought treatment showed the same pattern of shoot dry matter decrease of 33%. The lowest decrease of 11% was recorded from *Paenibacillus polymyxa* B-50; whereas the best inoculant *Bacillus thuringensis* BS-45 that induced the highest shoot dry matter of 2.4 g/pot showed a drastic decrease of 42% upon treatment of drought tolerance. Interestingly, the other best performer *Ochrobactrum* RS-72 showed a 38% decrease in shoot dry matter with a similar treatment. Even though the inoculation of *Klebsiella michiganensis* PS-2 produced the lowest shoot dry matter (1.4 g/pot), it showed a relatively low decrease (14%) in its response to the drought test. In general, all plants showed a significant increase by inoculation with all rhizobacterial species, the *Paenibacillus polymyxa* B-50; *Pseudomonas fluorescens* FB-49, performed better under drought conditions than the other groups.

Similarly, inoculation with individual strains also improved plant biomass SH, RL, leave numbers, SDW, and RDW. *P. fluorescent* resulted in the highest biomass increase in underwater restriction followed by *P. polymyxa*. *Klebsiella michiganensis* showed the least drought stress improvement (38.7 cm) in SH compared to the other singly inoculated plants but performed better compared to non-inoculated control treatment (Table 5.7 and Figure 5.4).

Table 5.7. Plant growth promotion in A. abyssinica treated with different PGPR individually and consortium under control and drought stress conditions

Species	Treatments	SH (cm)/pot	RL (cm)/pot	SDW (g)/pot	RDW (g)/pot	Shoot: root ratio
	Control	38.3 ± 2.9^{i}	$10.0\pm2.6^{i-k}$	0.9 ± 0.2^{jk}	0.12 ± 0.01^{jk}	7.50
	Control +D	31.7 ± 2.1^{j}	7.0 ± 2.6^{k}	0.6 ± 0.1^{k}	0.1 ± 0.02^{k}	6.00
Acinetobacter calcoaceticus	BS-27	50.7 ± 0.6^{d}	$14.0\pm2^{f-h}$	$1.9\pm0.4^{b-e}$	$0.4\pm0.06^{d-i}$	4.75
	BS-27 +D	$43.3\pm1.5^{f-h}$	$11.0\pm1^{h-j}$	$1.5\pm0.2^{e-i}$	$0.3\pm0.04^{g-j}$	5.00
Paenibacillus polymyxa	FB-50	52.0 ± 2.6^{d}	$17.7\pm1.5^{b-e}$	$1.9\pm0.3^{b-e}$	$0.4\pm0.11^{d-i}$	4.75
	FB-50 +D	46.3 ± 3.1^{ef}	$11.7\pm2.1^{h-j}$	$1.7\pm0.1^{g-j}$	$0.5\pm0.05^{g-i}$	3.40
Serratia marcescens	RS-65	46.7 ± 3.2^{ef}	$16.0\pm1^{d-f}$	$1.5\pm0.4^{e-i}$	$0.3\pm0.09^{e-i}$	5.00
	RS-65 +D	39.3 ± 2.5^{hi}	$11.0\pm2^{h-j}$	1.1 ± 0.2^{ij}	$0.2\pm0.05^{j-k}$	5.50
Pseudomonas fluorescens	FB-49	61.0 ± 1.7^{b}	20.3 ± 1.5^{ab}	2.2 ± 0.4^{bc}	$0.60.03^{bc}$	3.67
	FB-49 +D	51.7 ± 1.5^{d}	$18.7\pm1.5^{b-d}$	$1.8\pm0.1^{d-h}$	$0.55\pm0.07^{c-f}$	3.30
Ochrobactrum intermedium	RS-72	51.7 ± 2.1^{d}	$16.7\pm1.5^{c-f}$	$2.1\pm0.4^{b-d}$	0.5 ± 0.12^{cd}	4.20
	RS-72 +D	44.0 ± 2^{fg}	$11.3\pm1.5^{h-j}$	$1.3\pm0.1^{g-i}$	$0.4\pm0.1^{e-h}$	3.25
Pseudomonas putida	BS-19	48.3 ± 1.5^{de}	$14.0\pm1^{f-h}$	2.2 ± 0.3^{bc}	$0.5\pm0.14^{c-e}$	4.40
	BS-19 +D	$40.3\pm2.5^{g-i}$	10.3 ± 1.5^{ij}	$1.6\pm0.3^{d-h}$	$0.4\pm0.04^{d-f}$	4.00
Agrobacterium tumefaciens	RS-79	$43.3\pm2.5^{f-h}$	$12.7\pm2.1^{g-i}$	$1.9\pm0.3^{c-f}$	$0.4\pm0.11^{d-h}$	4.75
	RS-79 +D	39.3 ± 1.5^{hi}	8.7 ± 1.5^{jk}	$1.4\pm0.2^{f-i}$	$0.3\pm0.08^{g-j}$	4.67
Enterococcus gallinarum	PS-16	46.0 ± 2^{ef}	$11.0\pm1^{h-j}$	$1.8\pm0.5^{c-f}$	$0.3\pm0.12^{g-i}$	6.00
	PS-16 +D	39.7 ± 2.5^{hi}	8.4 ± 2.1^{jk}	$1.4\pm0.2^{g-i}$	$0.2\pm0.06^{g-j}$	7.00
Bacillus thuringiensis	BS-45	51.0 ± 2^{d}	$11.3\pm1.5^{h-j}$	2.4 ± 0.2^{b}	$0.4\pm0.15^{d-i}$	6.00
	BS-45 +D	$42.7\pm2.1^{f-h}$	$9.3\pm1.5^{i-k}$	$1.4\pm0.1^{g-i}$	$0.2\pm0.08^{h-k}$	7.00
Klebsiella michiganensis	PS-2	$41.3\pm1.5^{g-i}$	$10.0\pm1^{i-k}$	$1.4\pm0.1^{g-i}$	$0.2\pm0.07^{g-j}$	7.00
	PS-2 +D	38.7 ± 2.1^{i}	8.3 ± 1.5^{jk}	$1.2\pm0.1^{h-i}$	$0.2\pm0.02^{i-k}$	6.00
FB-50+BS-27 + BS-19 +FB-49	Consortia	70.3 ± 1.5^{a}	22.3 ± 2.1^{a}	2.9 ± 0.3^{a}	0.9 ± 0.12^{a}	3.20
	Consortia +D	57.3 ± 1.5^{c}	19.3 ± 2.5^{bc}	$2.1\pm0.2^{b-d}$	0.8 ± 0.07^{ab}	2.62

Means with the same letter down the column are not significantly different at ($p \leq 0.05$) by using DMRT. Mean ± SD (n = 3) and D represents drought. Consortia = FB-50 +BS-27 +BS-19 +FB-49. SH= shoot height, RL=root length, SDW= shoot dry weight, and RDW= root dry weight.

Mixed inoculations Single inoculation Control

Figure 5.4. Drought stress enhancements of PGPR isolates in acacia plants.

Consortia =FB-50 + BS-27 + BS-19 + FB-49 + D, single = FB-49 + D and control = without inoculation +D, represent water stress

5.4. Discussion

Degraded soil is a potential source of DS tolerant bacteria with 40% PEG with high OD reading. That is the reason why our PGPR strains could grow in a medium with reduced water content. The reason for drought tolerance in bacterial strains may be due to osmoregulation, the production of large chain EPS, and regulation of host stress response genes. *Pseudomonas fluorescent* FB-49 and *Pseudomonas putida* BS-19 best grow at PEG 40% (Table 5.2). Rhizobacterial species possesses high drought tolerant potentials with OD over 0.5 were selected following scale designed by Alikhani and Mohamadi (2010). Similarly, Kumar *et al.* (2014) reported that *Pseudomonas* and *Bacillus* sp. with good plant growth promoting ability which can survived 40.5% PEG. The isolates with the highest drought-tolerant ability showed also the highest biofilm formation at higher NaCl concentration (Figure 5.3). The survival and adaptation mechanisms to extreme drought conditions are previously indicated as biofilm formation (Kavamura *et al.*, 2013).

A dramatic change was observed in bacterial ability to form a biofilm both in 100 and 150 mM NaCl concentrations by PGPR isolates. This indicated biofilm formation was increased with increasing NaCl concentration (Figure 5.3). This can be attributed to the higher ionic strengths that are known to reduce the repulsion between a bacterial cell and a material surface, which are both typically negatively charged (Lionard *et al.*, 2012). Biofilm assists drought tolerance by producing extracellular matrices to maintain a hydrated root environment, increasing root-adhering soil, and stability (Sandhya *et al.*, 2009). Moreover, salt stress induces biofilm formation (Pan *et al.*, 2010). The formation of EPSs by rhizobacteria is one of the important mechanisms in exerting drought tolerance.

In this study, although the rhizobacteria showed a wider range of NaCl tolerance higher concentrations reduced their growth (Table 5.3). Different studies showed the high osmotic strength of some bacteria is due to the production of proline, glutamate, glycine, betaine, and trehalose in the cells (Anjum *et al.*, 2017). Among the tested strains, seven isolates were identified with high temperature (45°C) tolerance (Table 5.5). One possible reason for this could be the synthesis of heat shock proteins (Ali *et al.*, 2009). This could be attributed to the extremely resistant and dormant endospores produced by *Bacillus* spp. (Piggot and Hilbert, 2004). Sandhya *et al.* (2009) indicated that a strain of *Pseudomonas* AKM-P6 possessing plant growth-promoting properties enhanced tolerance of sorghum seedlings to high temperatures (47-50°C). Manasa *et al.* (2017) suggested that the rhizobacterial isolates RR-1, GGP-1, and GNR-1 were both tolerant to high temperature (45°C) and also exhibited multiple beneficial plant growth-promoting activities. Although *in vitro* temperature selection is not considered promising approach for field applications but high-temperature tolerance can be useful for isolating competitive PGPR in oscillating temperature in the fields (Hungria and Vargas, 2000).

Concerning heavy metal resistance, four isolates *P. polymyxa* strain FB-50, *A. calcoaceticus* stain BS-27, *P. putida* strain BS-19, and *P. fluorescens* strain FB-49 showed 100% tolerance to all HMs tested. This trend was similar to the tolerance of *Paenibacillus* spp and *B. thuringiensis* to HMs like Cd, Cu, and Zn (Rathnayake *et al.*, 2009). Surface binding/reduced uptake, increased efflux intracellular sequestration, enzyme detoxication, and active transport are among the proven mechanisms of tolerance (Nies, 1992).

This study proved that inoculation of PGPR strains alone or in consortia has significantly enhanced the growth of Acacia seedlings under normal and drought conditions (Table 5.7). Bacterial inoculation significantly increased the biomass exposed to drought stress. Almaghrabi *et al.* (2013) also showed that inoculation of plants with PGPR increased the growth rate/yield and fosters seedlings emergence in plants under greenhouse trails. This finding indicates that inoculation with *Klebsiella michiganensis* PS-2 indicated shoot dry matter increment and enhanced drought stress by 50% in Acacia as compared to the control. Giongo *et al.* (2010) showed that inoculation with *Klebsiella* sp increased in the dry matter of *Lupinus albescens* by 81%, compared to the non-inoculated control.

The possible mechanisms associated with PGPR-derived drought tolerance include alterations in host root system architecture, osmoregulation, management of oxidative stress, and transcriptional regulation of host stress response genes (Forni *et al.*, 2017; Vurukonda *et al.*, 2016). This study confirmed that acacia inoculated with *Bacillus thuringiensis* BS-45 induced the highest shoot dry matter. The application of *Bacillus subtilis* (BERA 71) turned out to be potentially beneficial in ameliorating the deleterious impact of salinity and drought in *Acacia gerrardii* plant (Hashem *et al.*, 2016). The application of *Acinetobacter calcoaceticus* BS-27 indicated a 0.6 g DS alleviation in acacia shoot dry matter compared to control. Liu *et al.* (2019) find drought enhancement in a deciduous tree of *Sambucus williamsii* via the inoculation of *Acinetobacter calcoaceticus* X128. described how the PGPR *Paenibacillus polymyxa* enhanced drought tolerance in *Arabidopsis thaliana* plants. The present study finds that inoculations of potential rhizobacterial isolates enhanced shoot dry matter of drought-stressed acacia plants in a range of 2.1- 1.1 g (Table 5.7). Timmusk and Wagner (1999) and (Manoj *et al.*, 2016) evaluated defense enzymes to check the possible mechanisms of action to ameliorate DS due to the inoculation of *P. putida* RA *Acinetobacter* and *Pseudomonas* enhanced shoot of drought challenged grapevines indicating PGP activity of phytobeneficial microbes (Rolli *et al.*, 2015). This study suggests the integrative use of a combination and/or single application of PGPR strains to be a promising and eco-friendly strategy for reducing moisture stress in plants.

5.5. Conclusion

This study revealed that PGPR strains recovered from degraded lands in Ethiopia have exhibited a promising drought, salt, acidity temperature, and heavy metals tolerant capacity. *P. fluorescent*, *P. putida*, *A. calcoaceticus*, *A. tumefaciens* and *B. thuringensis* are some of the bacterial strains considered highly tolerant (HT) to induce osmotic stress. Most of the bacterial isolates are biofilm formers which play protective roles under stressing conditions. Some PGPR strains such as *P. polymyxa*, *A. calcoaceticus*, *P. putida* and *P. fluorescens* enhanced drought stress tolerance in Acacia under greenhouse conditions. Mixed inoculation resulted in higher drought tolerance in comparison to single inoculation.

Chapter 6

6. Phytobeneficial Traits of Rhizobacteria Isolated from Degraded Soil and their Effect on Improving Seeds Germination of Acacia (*Acacia abyssinica* Hochst. ex Benth)

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Abstract

Microbes are important components of the soil that remarkably improve ecosystem functions and services, and plant production, particularly under degraded ecosystem. However, the effectiveness of these microbes to effectively perform these functions depends upon the inherent characteristics of individual species/strains under their specific environment. This, therefore, necessitates isolation and characterization of these microbes with their multiple plant growth-promoting traits such as the production of hydrogen cyanide (HCN), indole acetic acid (IAA), hydrolytic enzyme, siderophore, and ammonia. Isolates with multiple plant growth-promoting traits were chosen to evaluate their effects on acacia seeds germination. The data showed that 45% each was categorized as high phosphate solubilizer (HPS) and medium phosphate solubilizer (MPS) with that amount of free phosphorus released ranging from 195 to 373 µg/mL. *Acinetobacter* BS-27 and *Pantoea* BS-38 with SI 7 and 6 showed the highest solubilization indices (SI). Similarly, *Pseudomonas* FB-49 produced the maximum P and IAA with 373 and 659.07µg/mL, respectively. All the phosphate solubilizing isolates acquired up to five multiple plant growth-promoting traits. Thus, *Agrobacterium* RS-79 and *Pseudomonas* BS-26 showed all plant growth-promoting properties. Amongst the isolates, *Pseudomonas* BS-26 and *Pseudomonas* FB-49 induced the highest (100%) seed germination activity caused by similar vigor indices of 343.33 and 306.67, respectively. In general, the data showed that

degraded soil is a potential source for effective phosphate solubilizing bacteria with multiple plant growth-promoting traits to be used for seed germination assay.

Keywords: Hydrolytic enzymes, Multiple plant growth promotion, Phosphate solubilization, Seeds germination assay

6.1. Introduction

Microorganisms are important components of the ecosystem that play a very important role in the geochemical cycle of nutrient and ecosystem functioning (Widawati, 2018). It has been known that almost all land plants form interactions with the nearby microbiota in all stages of their growth which is associated mainly with their ability to release root exudates (Schillaci *et al.*, 2019). However, their activity is influenced by different environmental and human factors such as population explosion and climate change that cause soil degradation that, in turn, reduce the bacterial abundance and their activities, nutrient availability.

Since the earliest time, microbial utilization for plant improvements has been practiced with proven benefits (Bhattacharyya and Jha, 2012). The genera *Pseudomonas, Acinetobacter*, *Bacillus, Azospirillum, Paenibacillus, Serratia, Klebsiella, Azotobacter, Enterobacter,* and *Agrobacterium* are the most widely known PGPR in the soil (Borriss, 2011; Shivlata and Satyanarayana, 2017; Sivasakthi *et al.*, 2014). They are also known to enhance crop production.

These microbes enhance plant growth and stress tolerance through direct (phytohormone and siderophores production, N_2 fixation, phosphate solubilization, and iron sequestration) and indirect (antibiotics and lytic enzymes production, induced resistance, HCN production, and competition) mechanisms (Ahemad and Mulugeta Kibret, 2014; Glick, 2012; Hardoim *et al.*, 2015). According to Gebhardt (2015), degraded soils are characterized by a reduction in soil fertility, soil organic matter content, particularly availability of phosphorus (P). Daniels *et al.*, (2009), estimated that plants require approximately 30 μmol/L of phosphorus for maximum productivity, but only about 1 μmol/L is available in many soils that have been recognized as a major growth-limiting factor. The efficiency of applied phosphorus rarely exceeds 30% due to fixation in soil (Sharma *et al.*, 2013). Phosphate-solubilizing bacteria increase the bio-availability of phosphorus by releasing acid phosphatases and organic acids (Rodríguez and Fraga, 1999; Schillaci *et al.*, 2019). It is clearly indicated that the inoculation of PGPR can reduce the application of synthetic phosphorus fertilizer by 50% without affecting plant growth parameters (Yazdani *et al.*, 2009).

In general, PGPR improves plant health and promotes growth by increasing seedlings' emergence, vigor, and yield in several agriculturally and environmentally important plants (Walker *et al.*, 2012). They also play a pivotal role in the revegetation of degraded habitats (Ansari and Ahmad, 2018; Grönemeyer *et al.*, 2012). Hence, degraded areas call for the re-introduction of indigenous microbes to maintain soil quality and fertility (Dastager *et al.*, 2011).

Seed dormancy affects both seed germination and subsequent growth of seedlings by causing a substantial reduction in yield loss (Leubner-Metzger, 2006). One of the important approaches used to break dormancy in seeds is the application of PGPR. The genera of *Pseudomonas*, *Bacillus*, *Enterobacter*, *Azospirillum*, and *Acinetobacter* significantly increased seed germination and seedlings vigor (Ahemad and Mulugeta Kibret, 2014; Cakmakçi *et al.*, 2006). Mafia *et al.* (2009) had shown that *Pseudomonas* sp. and *Bacillus* sp., are the best rhizobacteria for seed germination and growth promotion of multipurpose trees like *Eucalyptus cloeziana* and *E. grandis*. The production of phytohormones such as gibberellins by *B. licheniformis* was attributed to increasing seed germination and seedlings growth promotion in *Acacia senegal* and *Prosopis cineraria* and hence facilitated fast rehabilitation of degraded habitats (Sunil *et al.*, 2011). This indicates that the practical application of PGPR as biofertilizers requires isolation and screening of multiple plant growth-promoting (PGP) traits to enhance adaptation of multipurpose trees such as Acacia species under nutrient and water deficient habitats.

Therefore, this study was initiated to assess the screening of effective rhizobacteria from degraded soil and evaluate their performance on re-introduction to improve seed germination efficiency and growth promotion on Acacia seedlings on degraded soils under greenhouse conditions.

6.2. Materials and Methods

6.2.1. Bacterial strains, Source and their Culture Preparations

Rhizobacterial isolates were isolated from the woody plants described above in Chapter 4 and deposited at culture collection of the Addis Ababa University at the Department of Microbial Cellular and Molecular (MCMB) (Alemayehu Getahun *et al.*, 2020a; Alemayehu Getahun *et al.*, 2020c). These strains were preliminarily screened for phosphate solubilization and the selected isolates (34) were further evaluated for their multiple plant growth-promoting properties. For every test, each strain was grown overnight in broth cultures from which 100 µL inoculum with approximately 1 x 10^8 CFU/ mL) was inoculated on appropriate media.

6.2.2. PGPR Screening for Plant growth promotion (PGP)

Seventy-three (73) isolates were screened to evaluate their ability to produce ammonia, hydrogen cyanide (HCN), indole acetic acid (IAA), siderophore, and phosphate solubilization, hydrolytic enzymes activities. Apart from that, they were tested for their ability to enhance seed germination using standard methods.

6.2.2.1. Production of Ammonia

The ability of isolates to produce ammonia was tested following the method of (Joseph *et al.*, 2012). The isolates were inoculated into 5 mL of peptone water (5 mL) and incubated at 30˚C for 4 days. The culture was then treated with 1 mL of Nessler’s reagent (Mercuric chloride (50 g in 50 mL in cold water, Potassium iodide (22 g in 350 mL of water, Sodium hydroxide (200 mL of 5N) prepared in 1 L solution) to detect the development of faint yellow and deep yellow to a brown color indicating ammonia production

6.2.2.2. Phosphate solubilization

The ability of the bacterial isolates to solubilize phosphate was evaluated according to the standard method (Premono *et al.*, 1996). The isolates were spot inoculated on Pikovaskaya's agar medium (plates) containing g/L; (glucose 10, tricalcium phosphate (TCP) 5, $(NH_4)_2SO_4$ 0.5, KCl 0.2, $MgSO_4$ 0.1, $MnSO_4$ trace, $FeSO_4$ trace, yeast extract 0.5 and agar 15). The plates were incubated at 30±2°C for 4-5 days to detect clear zone formation around colonies. The phosphate-solubilizing index (PSI) was measured in centimeters (cm) and calculated according to Sridevi and Mallaiah (2009);

$$\mathrm{PSI} = \frac{\text{Colony diameter} + \text{ Halozone diameter}}{\text{Colony diameter}}$$

The solubilization efficiency was also assessed based on the scale formulated by (Silva Filho and Vidor, 2000). PSI values less than 1.0 were classified as very low solubilizers (VLS), PSI between 1.0 and 2.0, PSI between 2.0 and 3.0, and PSI above 3.0 were categorized as low solubilizers (LS), medium solubilizers (MS), and high solubilizers (HS), respectively.

Quantitative estimation of solubilized phosphorus was done using vanadomolybdophosphoric yellow color method in pikovskaya's broth containing TCP (Wu *et al.*, 2012). Thus, 0.1 ml of bacterial culture (inoculum adjusted to 1×10^8 CFU/mL) was inoculated into ten milliliters of pikovskaya's broth and incubated at 28 ± 2°C up to 8 days. Then, 1 ml of the supernatant was taken and centrifuged was at 10,000 rpm for 20 min and was passed through a 0.45 mM Millipore filter, from which 0.1 ml of the filtrate was mixed with 0.25 mL of Barton's reagent with the following composition (25 g ammonium molybdate in 400 ml distilled water, 1.25 g ammonium metavanadate in 300 ml of boiling water, 250 mL concentrated HNO_3 and volume was made up to 5 mL with double distilled water. The mixture was left for 10 min, to read color change using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom) at 430 nm. The amount of P-solubilized was extrapolated from the standard curve plotted against known concentrations of potassium dihydrogen phosphate (KH_2PO_4) **(**0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0 mL.

6.2.2.3. Indole-3-Acetic Acid (IAA) Production

To test IAA production, 100 μL of each of the 48-h bacterial culture was inoculated in 5 mL nutrient broth medium in the presence of 500 μg/mL of L-tryptophan and incubated at 30±2°C for 48 h (Shaik *et al.*, 2016). The culture was centrifuged at 3000 rpm for 15 minutes from which, 2 mL of the supernatant was mixed with two drops of orthophosphoric acid and 4 mL of the Salkowski reagent (50 mL, 35% of perchloric acid, 1mL 0.5M $FeCl_3$ solution). The optical density (OD) at 535 nm of the mixture read using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom) to determine the intensity of color formation. The production of IAA was quantified against a standard curve was plotted with known IAA in the range of 0-100 µg/mL.

6.2.2.4. HCN production

HCN production was done both on nutrient agar and nutrient broth amended with 4.4 g/L of glycine and bacteria were streaked on the nutrient agar plates (Joseph *et al.*, 2012). For plate assay, a Whatsman filter paper soaked in the solution of 2% Na_2CO_3 and 0.5% picric acid solution was placed on the lid (on the top of the cover) of streaked plates and sealed with parafilm. Plates were incubated at 30±2°C for 4 days. The change in the color of filter paper from orange to red indicates HCN production.

In the tube test, 25 µL of the bacterial culture was inoculated into 5 ml of nutrient broth supplemented with 4.4 g/L of glycine. Uniform strips of filter paper strips (10 x 0.5 cm^2) were soaked in picric acid solution and inserted in half of the vials and tightened with a screw cap. Vials were sealed with parafilm and incubated for 72 h. Production of HCN was indicated by the change in color of the filter paper strips from yellow to brown to red. The intensity of the color was recorded visually. The color was eluted by placing the filter paper in a test tube containing 10 mL of distilled water and its absorbance was read at 625 nm (Sadasivam and Manickam, 1992).

6.2.2.5. Protease, Cellulase, Lipase, and Chitinases activity

The qualitative assay for protease production was performed on sterile skim milk agar plates (skim milk 15, yeast extract 0.5, agar 9.13) in g/L (Geetha *et al.*, 2014). Isolates were spot inoculated and incubated at 30°C and zones of clearance around the colony indicated the presence of protease activity. The cellulase activity of isolates was undertaken on carboxymethyl cellulose (CMC) agar plates with the following composition in g/L (CMC 2, $NaNO_3$ 1, K_2HPO_4 1, KCl 1, $MgSO_4$ 0.5, $FeSO_4$ 0.01, yeast extract 5 and agar 15) and incubated at 30°C for 2 days (Khianngam *et al.*, 2014). The plates were flooded with iodine to detect clear zone formation by the isolates.

The bacterial isolates were also spot inoculated on lipase medium composed of (g/L): peptone, 10; NaCl, 5; $CaCl_2$, 0.1; agar-agar, 20 and tween 20, 10 mL (v/v) incubated at 28°C for 48 h to detect their lipase activity (Kumari *et al.*, 2010). The qualitative assay for chitinase production by the isolates was performed on colloidal chitin agar (CCA) with the following components in g/L with some modification (Colloidal chitin 4.0, K_2 HPO_4 0.7, KH_2 PO_4 0.3, $MgSO_4$ 0.5, NaCl (0.3), yeast extract (0.2) $FeSO_4$ 0.01, $MnCl_2$ 0.001, Agar 20.0) for 5 to7 days at 30°C (Bansode and Bajekal, 2006). Development of clearance halo zone around the growing colony after the addition of iodine was considered as positive for chitinase enzyme production.

6.2.2.6. Siderophores production

The ability of isolates to produce siderophore was detected by growing them on Fiss Minimal Medium with the following compositions in g/L (5.03 KH_2PO_4, 5.03 L-asparagine, 5.0 glucose), 40 mg/L $MgSO_4$ and 500 µg/L $ZnCl_2$, 139 µg/L $FeSO_4$ was added to the final medium containing 0.5 µM iron) (Goswami *et al.* (2014). After 48 h of incubation, the cultures were centrifuged at 1000xg for 15 min and the supernatant was examined for siderophore production using $FeCl_3$ test (Jalal and van der Helm, 2017). Thus, 0.5 mL of culture filtrate was mixed with 0.5 mL of 2% aqueous $FeCl_3$ solution. The appearance of reddish-brown or orange color was a positive indication for siderophore production.

6.2.3. Germination assays of Acacia seeds

Seed's germination assay for 15 selected bacterial inoculants on acacia seeds was done via germination check of both root and shoot of the germinating seeds before pot experiment (Mia *et al.*, 2012). Seeds of *Acacia abyssinica* were collected from North Shewa Zone, Fiche, and were surface sterilized briefly with 70% ethanol and 1% sodium hypochlorite for 2 min, followed by repeated washing with sterile water. The seeds were placed in plates and then soaked in (10 mL) broth containing overnight cultured bacterial isolates (10^8 CFU/mL) and left for 5 hrs at 30°C. Seeds that were soaked in normal TSB were included as a control.

Seven seeds of each treatment were kept equidistant in sterilized Petri plates containing moist filter paper and cotton. Both treated and untreated seeds (plates) were arranged in Complete Randomized Design (CBD) in triplicates and then incubated at 28 ±2°C for 7 days. Seed's germination and percent seedlings emergence were calculated using the following formula;

$$\text{Germination Percentage (GP) \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \text{ x } 100$$

Then, the radicle and plumule length of germinated seeds were measured (Gholami *et al.*, 2009). The seed vigor index (VI) is calculated by determining the germination percentage and the total seedling length (mm) (radicle plus Plumule length) of the same seed lot.

$$\text{SeedVigor index (SVI)} = \%\,\text{germination} \times \text{Mean total plant length}$$

6.2.5. Data Analysis

The means and standard deviations of the data were calculated. Three replicates were used for each experiment. Analysis of Variance (ANOVA) was used to test for significant differences of measurements of each bioassay, whereas Duncan's Multiple Range Test (DMRT) was employed for mean separation among different treatments at $p \leq 0.05$. The Statistical Analysis System (SAS Ver. 9.0) was used for the analysis. Phylogenetic analysis of partial 16S rRNA gene sequences was done using Mega 7 software version 7.0.2.

6.3. Results

6.3.1. Plant Growth Promotion (PGP) traits

Totally 73 isolates were screened for their plant growth-promoting activities of which 38 (52.05%) were phosphate solubilizers. Based on the solubilization index (SI), four distinct groups of PS isolates were identified as high solubilizers (HS), medium solubilizers (MS), low solubilizers (LS), and very low solubilizers (VLS. Thus, seventeen isolates (46.58%) each were classified as HS (high solubilizers) and MS (medium solubilizers), groups, with SI values >3 and between 2.1 and 3, whereas 4.11% and 1.37% of the isolates were grouped into LS (Low solubilizers) and VLS, (very low solubilizers) with SI values between 1 and 1.9, respectively Table 6.1). but for further study, only 34 isolates possessing HS and MS SI values are chosen.

Table 6.1. Qualitative and quantitative phosphate solubilizing (PS) and IAA potential of PGPR strains isolated from acacia plants established in degraded soil after 8 days of incubation.

S. No.	Isolates	SI	Level of PS	Concentration of P µg/mL	Concentration of IAA (µg/ml)
1	*Bacillus* sp. Strain BS-22	3.6±0.68^{c-f}	HS	354.85±4^{d}	233.15±0.033^{l-p}
2	*Bacillus* sp. Strain BS-40	2.7±0.36^{h-k}	MS	323.03±3.5^{e}	-
3	*Bacillus* sp. Strain BS-47	3±0.36^{f-k}	MS	303.33±2^{f}	-
4	*Enterococcus* sp. Strain PS-4	2.8±0.53^{d-i}	MS	301.82±4^{f}	425.74±0.028^{d}
5	*Enterococcus* sp. Strain PS-5	3.33±0.46^{d-i}	HS	353.33±3.5^{d}	-
6	*Enterococcus* sp. Strain PS-9	2.6±0.3^{i-k}	MS	204.85±3^{e}	-
7	*Paenibacillus* sp. Strain BS-30	2.6±0.53^{i-k}	MS	321.52±4^{e}	62.78±0.021^{t}
8	*Agrobacterium* sp. Strain RS-79	3±0.2^{e-i}	MS	326.06±3^{e}	288.70±0.023^{f-i}
9	*Ochrobactrum* sp. Strain RS-67	3±0.36^{e-i}	MS	359.39±1^{cd}	-
10	*Ochrobactrum* sp. Strain RS-70	3±0.17^{e-i}	MS	320.00±2^{e}	92.41±0.015st
11	*Acinetobacter* sp. Stain BS-27	7±0.35^{a}	HS	350.30±2.5^{d}	270.19±0.032^{f-k}
12	*Pseudomonas* sp. Strain BS-19	3.34±0.4^{d-i}	HS	368.48±2.5^{ab}	359.07±0.021^{e}
13	*Pseudomonas* sp. Strain BS-21	3.33±0.55^{d-i}	HS	351.82±6^{d}	-
14	*Pseudomonas* sp. Strain BS-23	3.4±0.53^{e-h}	HS	323.03±4^{e}	351.67±0.020^{e}
15	*Pseudomonas* sp. Strain BS-24	3.66±0.38^{c-f}	HS	195.76±2^{n}	210.93±0.018^{n-q}
16	*Pseudomonas* sp. Strain BS-26	3±0.62^{e-k}	MS	213.94±4^{l}	255.37±0.028^{i-m}
17	*Pseudomonas* sp. Strain BS-28	4.25±0.2^{c}	HS	353.33±2.5^{d}	277.59±0.020^{f-j}
18	*Pseudomonas* sp. Strain BS-52	3.2±0.46^{e-j}	HS	368.48±2^{ab}	-
19	*Pseudomonas* sp. Strain BS-53	3±0.45^{e-i}	MS	288.18±2^{g}	436.85±0.031^{d}
20	*Pseudomonas* sp. Strain RS-75	2.86±0.36^{f-k}	MS	262.42±2.5^{i}	262.78±0.026^{f-l}
21	*Pseudomonas* sp. Strain FB-49	3.4±0.2^{c-g}	HS	373.03±3^{a}	659.07±0.052^{a}
22	*Klebsiella* sp. Strain PS-1	3.66±0.15^{c-e}	HS	201.82±1^{mn}	233.15±0.028^{k-o}
23	*Klebsiella* sp. Strain PS-3	3±0.36^{e-k}	MS	198.79±2^{mn}	-
24	*Morganella* sp. Strain PS-12	4±0.36^{cd}	HS	363.94±3^{bc}	240.56±0.040^{i-n}
25	*Pantoea* sp. Strain BS-48	3.4±0.36^{e-h}	HS	303.33±2^{f}	299.81±0.023^{f}
26	*Pantoea* sp. Strain BS-35	2.5±0.2^{jk}	MS	257.88±3^{ij}	177.59±0.015^{rq}
27	*Pantoea* sp. Strain BS-38	6±0.2^{b}	HS	363.94±2.5^{bc}	188.70±0.018^{p-q}
28	*Serratia* sp. Strain BS-20	3±0.3^{e-k}	MS	329.09±2.5^{k}	218.33±0.025^{m-p}
29	*Serratia* sp. Strain BS-42	2.4±0.1^{k}	MS	303.33±2^{f}	-
30	*Serratia* sp. Strain PS-54	3.5±0.5^{c-g}	HS	289.70±3^{g}	-
31	*Serratia* sp. Strain RS-65	2.5±0.2^{jk}	MS	285.15±2^{g}	88.70±0.026st
32	*Serratia* sp. Strain RS-73	3.66±0.12^{c-f}	HS	273.03±3^{h}	296.11±0.034^{fg}
33	*Serratia* sp. Strain RS- 57	4±0.46^{cd}	HS	351.82±2.5^{j}	-
34	*Serratia* sp. Strain RS-64	3±0.23^{e-j}	MS	300.30±2.5^{f}	107.22±0.058^{s}

HS- high solubilizers, MS- medium solubilizers, LS- low solubilizers, and VLS- very low solubilizer. Values are tabulated as means ± the standard deviation (SD), n=3. Values followed by the same letter within a row are not significantly different according to Duncan multiple range test at ($p \leq 0.05$).

The phosphate-solubilizing rhizobacteria were classified into 11 genera; *Pseudomonas* (10 strains), *Serratia* (7 strains), *Bacillus* (3 strains) *Enterococcus* (3 strains), *Pantoea* (3 strains), *Klebsiella* (2 strains) *Ochrobactrum* (2 strains), *Paenibacillus* (1 strain), *Agrobacterium*, (1 strain), *Acinetobacter* (1 strain), *and Morganella* (1 strain) (Table 6.1). *Pseudomonas and Serratia,* were the most diversified genera representing 30% and 21% of the isolates followed by and *Bacillus*, *Enterococcus*, *Pantoea* each representing 9% of the isolates, respectively. Interestingly, almost 50% of the isolates contributed to only 40% of the selected rhizobacteria.

Among the selected isolates, *Acinetobacter* BS-27 showed the highest SI (7) followed by *Pantoea* isolate BS-38 with SI (6), while *Morganella* strain PS-6 had the lowest solubilization (0.82) SI. The amount of solubilized phosphate they released was in the range of 195.76 - 373.03 µg/mL. The highest P solubilization was measured in strain *Pseudomonas* strain FB-49 (373.76 µg/ml) followed by *Pseudomonas* strain BS-19 (368.48 µg/mL) and *Pseudomonas* strain BS-52 (368.48 µg/mL), respectively.

6.3.1.2. Indole-3-Acetic Acid (IAA) Production

Among the selected isolates, 67.65% showed the ability to produce IAA ranging from 62.78(µg/ml) up to 659.07µg/mL) (Table 6.1). Maximum IAA production was recorded from *Pseudomonas* strain FB-49 (659.07µg/mL, followed by *Pseudomonas* strain BS-53 and *Enterococcus* strain PS-4 (436.85, 425.74 µg/ml) respectively.

6.3.1.3. Screening for Biocontrol traits

All the isolates were able to produce ammonia (data not shown); twenty-three (67.65%) and 24 (70.59%) of the isolates were able to produce hydroxamate type siderophore and HCN, respectively (Table 6.2). Of the 34 PS isolates, 14 isolates (41%) produced chitinase with the highest chitin degradation halo zone diameter of 4.75; and 30 isolates (88.24%) formed halo zones on skim milk agar with the highest protein degradation halo zone diameter of 4.52 (Table 6.2). Similarly, cellulase and lipase enzymes were detected from 27 (79.41%) and 26 (76.50%) of the isolates (Table 6.2).

Table 6.2. The antagonistic and other different plant growth-promoting properties (PGPR) of the selected rhizobacteria isolated from standing Acacia trees from degraded land from Central Ethiopia

Isolates	HCN	Siderophore	Chitinase activity halo zone	Protease activity	Cellulase activity	Lipase activity
Bacillus sp. Strain BS-22	+	-	3.73±0.42^{c-f}	4.52	+	+
Bacillus sp. Strain BS-40	-	+	2.5±0.5^{jk}	3.36	-	-
Bacillus sp. Strain BS-47	-	+	-	-	+	+
Enterococcus sp. Strain PS-4	+	+	1.2±0.21^{l}	2.65	+	-
Enterococcus sp. Strain PS-5	+	-	4.75±0.32^{a}	3.9	+	+
Enterococcus sp. Strain PS-9	+	+	-	2.44	+	+
Paenibacillus sp. Strain BS-30	-	+	-	2.61	-	-
Agrobacterium sp. Strain RS-79	+	+	3±0.36^{g-j}	2.47	+	+
Ochrobactrum sp. Strain RS-67	+	+	3.67±0.40^{c-g}	3.34	-	+
Ochrobactrum sp. Strain RS-70	+	+	-	2.47	+	+
Acinetobacter sp. Stain BS-27	+	+	3.67±0.32^{c-g}	2.04	+	-
Pseudomonas sp. Strain BS-19	+	+	-	2.33	+	+
Pseudomonas sp. Strain BS-21	+	+	-	2.68	+	+
Pseudomonas sp. Strain BS-23	+	-	-	2.61	+	+
Pseudomonas sp. Strain BS-24	+	-	-	2.53	+	+
Pseudomonas sp. Strain BS-26	+	+	3±0.3^{g-j}	2.15	+	+
Pseudomonas sp. Strain BS-28	-	+	3.87±0.31^{b-e}	2.74	+	+
Pseudomonas sp. Strain BS-52	-	+	-	2.66	+	-
Pseudomonas sp. Strain BS-53	-	-	-	2.26	-	+
Pseudomonas sp. Strain RS-75	-	+	-	3.07	+	+
Pseudomonas sp. Strain FB-49	+	+	3.9±0.2^{b-d}	3.45	+	+
Klebsiella sp. Strain PS-1	+	-	-	-	+	+
Klebsiella sp. Strain PS-3	-	-	-	-	+	+
Morganella sp. Strain PS-12	-	+	-	2.49	+	+
Pantoea sp. Strain BS-48	+	+	3.5±0.36^{d-h}	3.31	-	-
Pantoea sp. Strain BS-35	+	+	-	2.68	+	+
Pantoea sp. Strain BS-38	+	-	-	2.71	-	-
Serratia sp. Strain BS-20	+	-	3.5±0.56^{d-h}	2.38	+	+
Serratia sp. Strain BS-42	-	+	3.5±0.58^{d-h}	2.57	-	+
Serratia sp. Strain PS-54	+	-	-	2.35	+	+
Serratia sp. Strain RS-65	+	+	-	2.27	+	+
Serratia sp. Strain RS-73	+	+	2.25±0.21^{k}	2.52	+	+
Serratia sp. Strain RS- 57	+	-	-	-	+	-
Serratia sp. Strain RS-64	+	+	-	2.79	+	+
	24	23	14	30	27	26
	70.59	67.65	41	88.24	79.41	76.50

HCN = hydrogen cyanide, – = non producers, + = producers for the tested traits. Legend: data were presented as re mean ± SD from (n =3) replications and values followed by the same letter(s) indicate no significant difference (p > 0.05) at 95% confidence interval. **-** = non producers, + = producers for the tested traits

6.3.1.4. Multiple Plant Growth Promoting Traits

Isolates *Pseudomonas* sp. Strain BS-26 and *Pseudomonas* sp. Strain FB-49 and *Agrobacterium* RS-79 displayed all the PGP traits tested; followed by three strains; *Pseudomonas* sp. Strain BS-19, *Pseudomonas* sp. Strain BS-28, *Pseudomonas* sp. Strain RS-75, three strains; *Serratia* sp. Strain RS-65, *Serratia* sp. Strain RS-73 *and Serratia* sp. Strain RS-64, and one strain each from *Bacillus* sp. Strain BS-22, *Enterococcus* sp. Strain PS-4, *Ochrobactrum* sp. Strain RS-70, *Acinetobacter* sp. Stain BS-27, *Morganella* sp. Strain PS-12, *Pantoea* sp. Strain BS-35, (Table 6.3). In general, almost 50% of the *Pseudomonas* and *Serratia* spp displayed almost all of the multiple growth-promoting characters tested; whereas representatives of *Paenibacillus* sp., *Bacillus* sp. and *Klebsiella* sp. showed less PGP properties.

Table 6.3. Multiple PGP traits of the 34 phosphate solubilizing bacteria

Isolates	PS.	IAA	HCN	SP	Chitinase	Protease	CMC	Lipase	Ammonia	Total %
Bacillus sp. Strain BS-22	+	+	+	-	+	+	+	+	+	8
Bacillus sp. Strain BS-40	+	-	-	+	+	+	-	-	+	5
Bacillus sp. Strain BS-47	+	-	-	+	-	-	+	+	+	5
Enterococcus sp. Strain PS-4	+	+	+	+	+	+	+	-	+	8
Enterococcus sp. Strain PS-5	+	-	+	-	+	+	+	+	+	7
Enterococcus sp. Strain PS-9	+	-	+	+	-	+	+	+	+	7
Paenibacillus sp. Strain BS-30	+	+	-	+	-	+	-	-	+	5
Agrobacterium sp. Strain RS-79	+	+	+	+	+	+	+	+	+	9
Ochrobactrum sp. Strain RS-67	+	-	+	+	+	+	-	+	+	7
Ochrobactrum sp. Strain RS-70	+	+	+	+	-	+	+	+	+	8
Acinetobacter sp. Stain BS-27	+	+	+	+	+	+	+	-	+	8
Pseudomonas sp. Strain BS-19	+	+	+	+	-	+	+	+	+	8
Pseudomonas sp. Strain BS-21	+	-	+	+	-	+	+	+	+	7
Pseudomonas sp. Strain BS-23	+	+	+	-	-	+	+	+	+	7
Pseudomonas sp. Strain BS-24	+	+	+	-	-	+	+	+	+	7
Pseudomonas sp. Strain BS-26	+	+	+	+	+	+	+	+	+	9
Pseudomonas sp. Strain BS-28	+	+	-	+	+	+	+	+	+	8
Pseudomonas sp. Strain BS-52	+	-	-	+	-	+	+	-	+	5
Pseudomonas sp. Strain BS-53	+	+	-	-	-	+	-	+	+	5
Pseudomonas sp. Strain RS-75	+	+	+	+	-	+	+	+	+	8
Pseudomonas sp. Strain FB-49	+	+	+	+	+	+	+	+	+	9
Klebsiella sp. Strain PS-1	+	+	-	-	-	-	+	+	+	5
Klebsiella sp. Strain PS-3	+	-	-	-	-	-	+	+	+	5
Morganella sp. Strain PS-12	+	+	+	+	-	+	+	+	+	8
Pantoea sp. Strain BS-48	+	+	+	+	+	+	-	-	+	7
Pantoea sp. Strain BS-35	+	+	+	+	-	+	+	+	+	8
Pantoea sp. Strain BS-38	+	+	+	-	-	+	-	-	+	5

Serratia sp. Strain BS-20	+	+	-	-	+	+	+	+	+	7
Serratia sp. Strain BS-42	+	-	+	+	+	+	-	+	+	7
Serratia sp. Strain PS-54	+	-	+	-	-	+	+	+	+	6
Serratia sp. Strain RS-65	+	+	+	+	-	+	+	+	+	8
Serratia sp. Strain RS-73	+	+	+	+	+	+	+	+	+	8
Serratia sp. Strain RS- 57	+	-	+	-	-	-	+	-	+	5
Serratia sp. Strain RS-64	+	+	+	+	-	+	+	+	+	8
%	100	67.6	70.6	67.6	41.2	88.2	79.4	76.5	100	

PS- phosphate solubilizer, SP- siderophore production, CMC- carboxymethyl cellulose, - = negative (non-producers), + = positive (producers), % = isolates showed positive response

6.3.1.5. Germination assay

Of the 34 PS isolates, 16 isolates with MPGP traits were examined for seeds germination assay and showed a significant difference in germination percentage, root and shoot length and seed vigor index compared to control (Table 6.4). The highest (100%) seed germination was observed with the treatment of *Pseudomonas* BS-26 and *Pseudomonas* FB-49 compared to 71% germination rate with the treatment of *Acinetobacter* BS-27. They showed a similar pattern in seedling vigor index (SVI) of 343.33 and 306.67), respectively, followed by inoculation of seeds with *Acinetobacter* BS-27 and *Pseudomonas* BS-28 with SVI of 176.17 and 104.76 ($p \leq 0.05$) compared to the uninoculated control (38.31). Similarly, the highest plumule and radicle length increments were observed with inoculated seeds with *Pseudomonas* BS-26 (2.37 and 1.07 cm) compared to uninoculated once (0.57 and 0.33 cm; Table 6.4).

Table 6.4. Effects of bacteria inoculation on acacia seed germination assay

Isolate	Plumule lengtl (cm)	Radicle lengtl (cm)	Total length (cm)	% Germinatior	SVI
Pseudomonas BS-19	1.27±0.33^{bcde}	0.50±0.06^{bcde}	1.77^{bcd}	57.14	100.95
Acinetobacter BS-27	1.73±0.15^{abcd}	0.73±0.15^{abc}	2.47^{abc}	71.42	176.17
Ochrobactrum RS-72	1.13±0.44^{cde}	0.33±0.09^{cde}	1.47^{cd}	42.86	62.86
Bacillus BS-22	0.40±0.40^{ef}	0.17±0.17^{de}	0.57^{ef}	14.29	8.10
Serratia RS-65	0.60±0.32^{ef}	0.20±0.12^{de}	0.80^{ef}	28.57	22.86

Enterococcus PS-5	0.60±0.60^{ef}	0.17±0.17^{de}	0.77^{ef}	14.29	10.96
Pseudomonas BS-26	2.37±0.45^{a}	1.07±0.18^{a}	3.43^{a}	100.00	343.33
Pantoea BS-35	0.84±0.09^{def}	0.31±0.14^{de}	1.15^{def}	28.57	32.95
Serratia RS-73	0.63±0.35^{ef}	0.23±0.15^{de}	0.87^{ef}	28.57	24.76
Pseudomonas BS-28	1.27±0.38^{bcde}	0.57±0.22^{bcd}	1.83^{bc}	57.14	104.76
Serratia BS-20	1.23±0.15^{bcde}	0.53±0.29^{bcd}	1.77^{bcd}	57.14	100.95
Agrobacterium RS-79	0.93±0.47^{cdef}	0.50±0.26^{bcde}	1.43^{cd}	14.29	20.48
Enterococcus PS-9	0.77±0.23^{ef}	0.43±0.12^{cde}	1.20^{de}	42.86	51.43
Enterococcus PS-4	0.47±0.26^{ef}	0.17±0.09^{de}	0.63^{ef}	28.57	18.09
Pseudomonas FB-49	2.10±0.15^{ab}	0.97±0.15^{a}	3.07^{ab}	100.00	306.67
Serratia RS-64	1.10±0.21^{cdef}	0.37±0.09^{cde}	1.47^{cde}	42.57	62.44
Control	0.57±0.35^{ef}	0.33±0.18^{cde}	0.90^{ef}	42.57	38.31

Mean ± SD values followed by the same letter within the row are not significantly different according to Duncan's multiple range test at ($p \leq 0.05$). SVI = Seed vigor index

The application of PGPR visibly increased the plumule height and radicle length in the germination assay of acacia seeds compared to un-inoculated one (Figure 6.1).

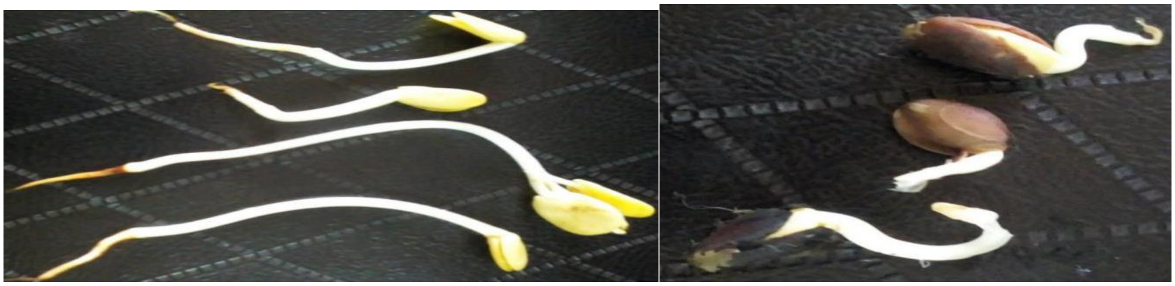

Figure 6.1. Effect of PGPR strains on germination (root and shoot length) in inoculated (left) and control (right) seedlings of acacia.

6.4. Discussion

The study revealed that of the 34 PS genera, *Pseudomonas* spp and *Serratia* spp. were the dominant (50% of the isolates were phosphate solubilizers). Similarly, based on their solubilization potential, 45% each isolate was grouped as high and medium PS with SI of 3.2 to 7 and 2.4 to 3, respectively. The variable potential of phosphate solubilization based on SI on an agar plate in the present study may be because of the varying type, amount, and diffusion rates of diverse organic acids secreted by rhizobacterial isolates. Batool and Iqbal (2018) showed P solubilization capacity of PGPR with 4 to 7 SI. Diriba Muleta *et al*. (2013) also reported phosphate solubilizing rhizobacteria isolated from the natural coffee forest in southwestern Ethiopia with PSI values between 2.05 and 5.85. The presence of a clear zone indicates P solubilization and is mainly related to the production of organic acids that can form metal complexes with calcium, magnesium, and iron thereby converting into soluble forms (Bashan *et al.*, 2013).

Rhizobial isolates that have shown higher SI on solid agar medium did not show a similar trend in liquid broth medium. Some isolates showed larger clear zones on agar but low phosphate solubilization in a liquid medium. This shows that the production of higher SI on a solid medium does not necessarily show solubilization efficiency in a liquid medium. This might be due to the type and number of organic acids released by the isolates and some isolates with the little clear zone on solid agar medium exhibited higher efficiency for dissolving insoluble phosphates in a liquid medium. Firew Elias *et al.* (2016) also reported a similar trend in fungal isolates from plants in Jimma Zone, Southwest Ethiopia. Phosphate solubilizing bacteria during the processes of chelation, acidification, and exchange reactions can avail insoluble phosphate into available forms for plants to increase crop yield (Pandey *et al.*, 2015).

The study revealed that 68% of the rhizobacterial isolates produced IAA and the quantity produced showed variations ($p \leq 0.05$). Sahay *et al.* (2012) reported that 50% of IAA producing bacterial isolates from Sambhar Lake, an extreme hypersaline environment of India. The higher IAA production was observed in *Pseudomonas fluorescens* strains FB-49. Variation in IAA production is most probably due to the inherent characteristics of the bacterial strain, culture condition, growth stage, and substrate availability, and environmental conditions (Pant and Agrawal, 2014). The data showed that 80% of the *Pseudomonas* isolates were capable of producing indole acetic acid (Table 6.1). Similarly, Aly *et al.* (2012) revealed the production of IAA by *Azotobacter* and *Pseudomonas* species. The maximum production in *Pseudomonas* species is due to the biochemical mechanism and pathway of IAA synthesis (Saharan and Nehra, 2011).

In this study only one isolate, *Bacillus* sp. Strain BS-22 produced 233 IAA (µg/ml) which was much lower than the ones produced by strains of *B. pumilus* and *B. licheniformis* to the tune of 445.5 and 335 µg/ml IAA (Singh *et al.*, 2011). IAA producers are potential plant growth stimulators provided that tryptophan, the precursor of IAA is available and the amount of IAA meets the plant requirement (Cruz and Cadiente, 2016). Different bacterial groups were reported to produce IAA and involve cell expansion, gene regulation, nutrient uptake, and root proliferation, improved seed germination, and stimulated orchid development (Francis *et al.*, 2010; Pandey *et al.*, 2015).

Twenty-three (67.65%) and 24 (70.59%) of the isolates were able to produce hydroxamate type siderophore and HCN respectively (Table 6.2). The production of siderophore and HCN are some of the indirect PGP traits found among the PGPR implicated with plant protection and a desirable trait to promote plant growth to induce plant resistance to stresses (Ngoma *et al.*, 2013). The presence of HCN in the soil can also act as an efficient biological weed control measure by inhibiting seed germination and seedling vigor (Banerjee *et al.*, 2010).

All bacterial isolates produce ammonia which is another important trait that indirectly influences plant growth. The accumulation of ammonia in soil may increase in pH by creating alkaline conditions of soil at pH 9 - 9.5. This suppresses the growth of certain fungi and nitrobacteria as it has a potent inhibition effect. It also disturbs the equilibrium of the microbial community and inhibits the germination of spores of many fungi (Swamy *et al*., 2016). The previous finding also indicated that inoculation of ammonia producing PGPR increases the root and shoot length and biomass of maize; *Z. mays* (Marques *et al.*, 2010).

Additional biocontrol trait is siderophores production (64%) which was verified by the development of orange color with tetrazolium assay as indicated previously (Table 6.2). Hydroxamate type siderophores production was reported by *P. fluorescens, Magnetospirillum magneticum* and root nodulating bacteria such as genus *Rhizobium* (Ali and Vidhale, 2013; Calugay *et al.*, 2003). Siderophore secreting bacteria help in the transport of Fe^{3+} inside the plant cell and contribute to plant growth and productivity (Pandey *et al.*, 2015). As a chelating agent, siderophore producers deprived off iron from pathogenic fungi because of the higher affinity of bacteria to iron, and that indirectly facilitates plant growth promotion (Compant et al., 2005). Rhizobacterial strains produce a diverse range of siderophores like catechol, carboxylate, and hydroxamate types that have a higher affinity for iron (Ashish *et al.*, 2016; Kumar *et al.*, 2017). *Bacillus* spp and *Pseudomonas* spp are also reported as potential biofertilizers due to their siderophores production ability (Bjelić *et al.*, 2018).

The hydrolytic activities of the isolates were indicated in, proteases (91%), chitinases (41%), cellulase (79%), and lipase (77%) activities (Table 6.2). The production of lytic enzymes is a very important mechanism for cell wall lysis that digests or deforms components of the cell wall of fungal pathogens. PGPR control plant pathogens by producing growth inhibitors that could damage pathogen cells, removing pathogen-specific nutrients (competition), parasitism, and inducing resistance against the pathogen in the plant body. These potential bacteria could damage pathogens by producing lytic enzymes, antibiotics, and siderophores oriented iron chelation that ultimately excludes the pathogen from the niche. Velu (2013) reported that *P. fluorescens* produced more protease, gelatinase, and cellulase enzymes than other enzymes such as amylase and pectinase.

Several bacterial genera including *Bacillus, Enterobacter, Ochrobactrum, Pseudomonas, Serratia, Klebsiella*, and *Acinetobacter* are well-known antagonistic bacteria to combat plant pathogens (Berg, 2009; Tariq *et al.*, 2010). *Pseudomonas* spp produces lytic enzymes with pronounced antifungal activity especially proteolytic enzymes that play a role in plant growth promotion of apple and pearl (Ruchi *et al.*, 2012). *Bacillus* species are also found to secret several hydrolytic enzymes such as protease, chitinase, and cellulase which have a vital role in plant growth promotion and plant disease management (Kumar and D AP, 2012).

Antagonistic bacteria such as *Serratia marcescens* reduce the mycelial network of *Sclerotium rolfsii* by expressing chitinase. The consortia of lytic enzyme-producing rhizobacteria were used in the field by expressing a potent synergistic inhibitory effect against pathogens and promote plant growth (Someya *et al.*, 2007). Generally, hydrolytic enzymes directly contribute to the parasitization of plant pathogens and rescue plants from biotic stresses, and could enhance plant growth.

The rhizobacterial isolates possess multiple plant growth-promoting characters ranging from 5-10 traits under *in vitro* conditions (Table 6.3). Of the 34 phosphate solubilizers, 16 (47.05%) of them exhibited greater than 70.59% multiple PGP traits by revealing at least 7-9 positive results for the tested traits. Such multiple modes of action have been reported to exhibit more than three PGP traits, which may promote plant growth directly or indirectly or synergistically. Multiple PGP activities among PGPR have also been reported by other workers (Ahmad *et al.*, 2008; de Freitas *et al.*, 2007).

In this study, *Pseudomonas* BS-26 and *Pseudomonas* FB-49 were considered as potential strains in increasing acacia seed germination and vigor index. These isolates would have triggered the activity of specific enzymes that promoted early germination such as amylase, which has brought an increase in the availability of starch assimilation (Nezarat and Gholami, 2009). In another study, the highest seed germination percentage of *Eucalyptus grandis* was caused when inoculated with *Pseudomonas* *fulva* compared to the control treatment (Mafia *et al.*, 2009). The improvement in seed germination by PGPR was also found in *Crataegus pseudoheterophylla* Fatemeh *et al*. (2014), *Acacia senegal* Sunil *et al.* (2011), *Rosa damascene* Kazaz *et al*. (2010), and *Abies* spp. (Zulueta-Rodríguez *et al.*, 2015). The germination increase may be due to the increased synthesis of hormones (Ahemad and Khan, 2012).

Patten and Glick (2002) reported that the PGPR bacteria may enhance the growth of radicle of seedlings by inducing phytohormones production such as auxins (usually IAA). Also, significant promotion in root and shoot vigor could be occurred by a better synthesis of auxins. Vigor index reflects the health of the seedlings produced and so it takes into account the germination percent and radical length. Accordingly, high vigor index value indicates better seedlings' health. Generally, the use of phytobeneficial rhizobacteria can be considered as an excellent treatment to overcome seed dormancy as reported earlier in the case of hard-coated seeds (Yadav *et al.*, 2010).

6.5. Conclusion

The present study indicated that degraded soil is a source of potential PGPR with multiple plant growth-promoting (PGP) traits (P-solubilization, HCN and IAA production, ammonia production, and hydrolytic enzyme and siderophore production). *Pseudomonas* species are the dominant phosphate solubilizers followed by *Serratia* species. *Klebsiella* PS-2 and *Pseudomonas* FB-49 had the highest seed germination, shoot, and root length, and seed vigor index. The presence of various PGP traits in the strains may be the possible reason to protect the plant from various stresses.

Chapter 7

7.The effect of Bacterial Inoculation and Organic Amendment for Establishment of Some Multipurpose Trees on Degraded Land

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Abstract

Plantation of trees is one of the important approaches to rehabilitate degraded soil. To this end, organic amendment of soil and bio-augmentation with microorganisms play an important role in enhancing plant growth and plant establishment in the field. Hence, this study was initiated to assess the role of organic amendments (OAs) and bacterial inoculations (BI) on tree establishment on degraded soil under field conditions. Thus, six plots measuring 41 m x 4 m were established on which the soil was amended with organic matter and bacterial inoculation (BI) of the transplanted plants quarterly which were followed by monthly bacterial counts and measurement of plant growth parameters and survival rate of the plants for one year. The highest bacterial counts of (21.66), actinomycetes (2.29), and fungal (0.82) x 10^5 colony-forming unit g^{-1} soil were recorded on plots that received OAs. Also, there were significant ($p \leq 0.05$) increases in stem height (1, 0.42, and 0.39 m/plant), girths (12.0, 2.28, and 11.33 cm/plant), and branch numbers (5, 4, and 4.3/plant) on transplanted apple, acacia and prunus trees, respectively compared to the control. The highest survival rate was observed on an apple tree (*Malus domestica*) (80%) followed by acacia *(Acacia abyssinica)* (66%). The data, in general, showed that using OAs, and BI enhance the growth and establishment of trees for the successful rehabilitation of degraded land.

Keyword: Acacia, Bio-augmentation, Bacterial counts, Rehabilitation, Survival rate, Tree

7.1. Introduction

Microbes are an integral component of the soil ecosystem that enhances plant growth and health (Shanmugam *et al.,* 2017). Microbial diversity is a driver of soil ecosystem services that are influenced by different environmental factors. However, land degradation decreases soil fertility and reduces soil microbial biomass and activity due to human and nature-induced activities all over the world (Mhete *et al.*, 2020).

Land degradation is a problem that requires an urgent need for a proper restoration program with the plantation of different plants. Multipurpose trees can serve as an important tool for ecological restoration and land reclamation plans and amelioration of environmental problems (Tognetti *et al.*, 2013). The introduction of plants and shrubs species in degraded soils is a difficult task due to low soil fertility, a severe drought period, and other associated stresses (Caravaca *et al.*, 2002). The decline in soil fertility, mainly due to the inadequate organic matter levels in the soil, seems to be the most significant factor for the decreased sustainability of the system. Plant establishment can be successfully implemented with a follow up of starting from seed germination, seedling recruitment to proper transplantation. Seed treatment with PGPR has been used to reduce germination time and to increase the growth of various crops such as vegetables (Kisiel and Kępczyńska, 2016; Zhang *et al.*, 2016).

The re-establishment of indigenous tree species is an essential approach for improving degraded land (Mengual *et al.*, 2014). Transplanted plants can be established in the presence of diverse microbial flora such as mycorrhiza, rhizobacteria to rehabilitate degraded lands. However, rehabilitation with plantation of multi-purpose plants depending upon native microflora is very slow in a highly degraded soil as the topsoil has lost soil fertility and its plant-associated microorganisms (Ramachandran and Radhapriya, 2016). This needs reintroduction of organic amendments and phytobeneficial soil bacteria to recover the loss of soil fertility and microbial communities for establishment of these plants on degraded soils (Bashan *et al.*, 2012).

Applications of organic amendments (OAs) can cause changes in the physical, chemical, and biological properties of soils on degraded land There are different types of OAs that include manure, compost, biochar, and sewage sludge with proven increased microbial activity and improved soil fertility (Wu *et al.*, 2017). It provides essential nutrients necessary for rebuilding soil organic matter content and establishment of the microbial population (Bohre and Chaubey, 2014). Several authors have found that organic inputs increase soil microbial biomass and enhance microbial activity (Gu *et al.*, 2009; Hungria *et al.*, 2009).

All taken together, revegetation requires the addition of large amounts of organic amendments and phytobeneficial microbes to enhance the rehabilitation of degraded lands (Iverson and Maier, 2009). Soil microbial community abundance, diversity, and activity have been used as indicators of overall soil health and productivity of plants of which soil bacteria are the most dominant group (Alberton & Hungria, 2010; Mbuthia et al., 2015).

Soil bacteria are so valued because they have the highest abundance and widest distribution in soils Shanmugam *et al.* (2017) and play important roles in the decomposition of organic matter and release of mineral nutrients. Their diversity and activity may regulate the health and stability of soil ecosystems. Therefore, understanding and utilizing soil bacterial communities is of great potential significance for maintaining ecosystem resilience and promoting sustainable practices on degraded lands (Cookson *et al.*, 2008; Zhang *et al.*, 2019).

Organic amendments have long history in the field of agriculture but not in the field of rehabilitation of degraded soil. In addition to this, the information on organic amendments and microbial inoculations for the rehabilitation of degraded lands is very scarce (Maestre *et al.*, 2017). This study hypothesized that some multipurpose trees that are essential for revegetation of degraded lands would respond in the field to inoculation of selected bacteria and addition of and OAs. Therefore, the present study was aimed at evaluating the response of degraded land to bacterial inoculations in the presence of organic amendments during the field establishment of some multipurpose trees.

7.2. Material and Methods

7.2.1. Description of the study site

The study was conducted on marginal, abandoned, and degraded soil in North Shewa Zone, Oromia National Regional State, Ethiopia. The site is located at 9˚08′ 52″ N and 38˚56’ 13" E and an altitude of 3100 m above sea level for one year from March 2017 to February 2018. The soil of the experimental area was sandy clay loamy in texture (>50% clay). The soil pH was 5.69 with a soil salinity of 0.2 dS/m (Alemayehu Getahun *et al.*, 2020b). In the study area, heavy rain starts in June and ends in September and the dry season occurs from October to January which is followed by a short rainy season from February to May.

7.2.2. Sources of Microorganisms and multi-purpose Plants

Rhizobacterial isolates were isolated from the woody plants grown in the study area as described above in Chapter 4 and deposited at culture collection of the Addis Ababa University at the Department of Microbial Cellular and Molecular (MCMB). In this study, *Pseudomonas fluorescent* FB-49, *Pseudomonas putida* BS-19, *Paenibacillus polymyxa* BS-50, and *Acinetobacter calcoaceticus* BS-27 Alemayehu Getahun *et al.* (2020c) were used for field application. The multi-purpose plant species: Acacia (*A. abyssinica*), Apple (*M. domestica*), and prunus (*P. africana*), and Coffee (*C. arabica*) were selected. Acacia is the dominant tree species growing in the area and was recruited in the nursery near the plot. Apple tree was bought from the Girar Jarso farmers’ training center (FTC) found near the study area. Seedlings of *Prunus* were obtained from the Ethiopian

Biodiversity Institute (EBI) and coffee seedlings were kindly received from a farmer near the study area.

7.2.3. Experimental Design, Field Preparation and OAs Application

A total of 984 m^2 (~0.1 ha) of the plot was plowed and fenced for the field experiment. Experimental plots were prepared manually and the treatments were arranged in a completely randomized block design (CRBD) in five blocks perpendicular to the control placed upright of the terrain to prevent mix up of the treatments. 41 x 4 m plots separated by 1 m walkways. The different OAs used included farmyard manure (FYM), compost (PC), biochar (BC), bacterial inoculation (BI), BI x OAs i.e., combination of FYM, BC and PC and PGPR (0.5:1:1 100 mL per plant) with the corresponding dosage as indicated in Table 7.1, and control (CTRL). The amendments were manually mixed into the topsoil at a concentration of 0.5% (wt. %) in each plot. Microbial counts were made from each plot after application of the inputs and transplantation of *A. abyssinica*, *M. domestica* and *P. africana* for one year according to Ramachandran and Radhapriya (2016).

7.2.3.1. Treatments

The consortium contained 10^8 CFU mL^{-1} of PGPR inoculation (100 mL per plant) was done during transplanting and at 3 months intervals after multipurpose trees planting for 12 months. The bacteria were selected and used in the degraded land rehabilitation for multipurpose tree plantation. Each block consisted of 6 treatments (columns) per species with 10 plants per treatment (6 treatments x 10 plants per treatment x 3 plant species = 180 trees). Since coffee seedlings couldn't adapt to the environment, they were excluded from the experiment. Seedlings for transplantation to the field plots were of similar height: 45 cm for apple, 13 cm for acacia, and 18 cm for prunus. During the field experimental trial, irrigation was done by adding 1 L of tap water to each planting hole and covered with wheat straw to reduce evaporation.

Table 7.1. Type of OAs, sources, and doses used for the experiment

Types of OAs	Source	Dose (tonnes/ha)	Dose (kg/plot)
Biochar (BC)	Coffee husk	2	300
Compost (CP)	Floriculture	5	800
Farm Yard Manure (FYM)	Dried cow dung	5	800
BI	Isolated from the site	20 mL/plant	100 mL/plant
BI x OAs	BC: CP: FMY (1:1:1)	5	130:260:260:50 mL
Control (CTRL)	No input	-	-

7.2.3. Plate counts of cultivable microorganisms

The total number of cultivable bacteria, fungi, and actinomyces were counted on nutrient agar (NA), actinomyces isolation ager (AIA), and rose Bengal agar plates for the enumeration of bacteria, actinomyces, and fungi, respectively using the serial dilution plate method (Xu and Zheng, 1986).

7.2.3.1. Enumeration of Bacterial Populations

Soil samples from each plot were collected and prepared to serial dilution agar plate method, and inoculated into nutrient agar plates, and incubated at 28 ± 2°C for 24 h to 48h for the enumeration of soil bacteria. The bacterial counts were expressed as CFU g^{-1} of soil. All the tests were done in triplicate.

7.2.3.2. Enumeration of Actinomycetes population

One gram of soil from each site was suspended in 9 mL of sterile distilled water and further prepared to appropriate dilution from which 0.1 mL of the suspension was spread on the actinomycetes isolation agar (AIA) amended with nalidixic acid 100 mg/L and actidione 20 mg/L to minimize the bacterial and fungal growth respectively. Then the plates were incubated at 30°C for 10 days to calculate CFU g^{-1} of soil.

7.2.3.3. Fungal population count

For fungal population counts, the diluted soil samples were inoculated into Rose Bengal Agar medium supplemented with streptomycin sulfate (0.05 mg/L and incubated at 25 ±1°C for 3-5 days. Colony-forming units; were estimated in CFU g^{-1} of soil.

7.2.4. Measurement of plant parameters

For this experiment, a non-destructive method of assessing plant parameters was taken. These were plant height, trunk (stem) diameter (0.5 to 5 cm above soil level Bowers and Turner (2001), and the number of developing branches that were determined periodically after 3, 6, 9, and 12 months after planting (Ohsowski *et al.*, 2016). Finally, the survival rate of each tree was assessed by recording dead and alive plants after one year and the percentage of survived plants was calculated using the formula (Glenn *et al.*, 2001):

$$\text{The survival rate of trees} = \frac{\text{Number of survived plants}}{\text{Total number of transplanted trees}} \text{x } 100$$

7.2.5. Statistical Analysis

Microbial abundance and plant growth parameters were subjected to analysis of variance (ANOVA) (Duncan's test) to test the significant differences between the study plots. All the results were reported as the means ± standard deviation. Percentage analysis was done to determine tree survival rate and overall microbial abundance of the treatments. All statistical analyses were performed using SAS 9.0 software package (Bashan *et al.*, 2012).

7.3. Results

7.3.1. Effect of different amendments on soil microbial population

7.3.1.1. The Percentage and Total Microbial Abundance

In the present study, the total average bacterial count was highest (87%) in all plots followed by actinomycetes 10% and fungi 3% (Figure 7.1). The result showed that a one-time amendment with biochar, compost, and manure remarkably increased bacterial abundance compared to that of fungi.

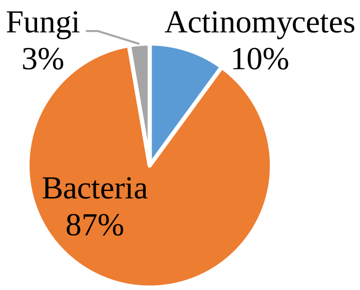


Figure 7.1. The percentage coverage of the overall microbial counts in the amended plots

All the microbial populations significantly increased on organically amended plots than the control plots (Figure 7.2). The average actinomycetes, bacterial and fungal counts from biochar amended soil were 2.01, 18.86, and 0.59 x 10^5, respectively. The bacterial abundance showed a significant ($p \leq 0.05$) difference across OAs but, there was no significant difference between fungal and actinomycetes counts in each treatment (Figure 7.2). Similarly, the average actinomycetes, bacterial and fungal counts from compost amended soil were 1.89, 16.96, and 0.53 x 10^5, respectively. There is a significant ($p \leq 0.05$) difference in bacterial abundance compared to actinomycetes and fungal counts (Figure 7.2). The average actinomycetes, bacterial and fungal counts in manure amended soil were 1.89, 16.91 and 0.57 x 10^5, respectively. Compost amended soil also showed a similar pattern in microbial counts.

The highest abundance was observed in a mixed amended plot. Thus, the average actinomycetes, bacterial and fungal counts were 2.29, 21.66, and 0.82 x 10^5, respectively (Figure 7.2). Likewise, the average actinomycetes, bacterial and fungal counts in nonamended (control) plots were 0.77, 3.0 and 0.39 x 10^5, respectively. The bacterial abundance was still higher and significant ($p \leq 0.05$) to actinomycetes and fungal counts. The microbial counts showed bacteria > actinomycetes > fungi in all the amended plots.

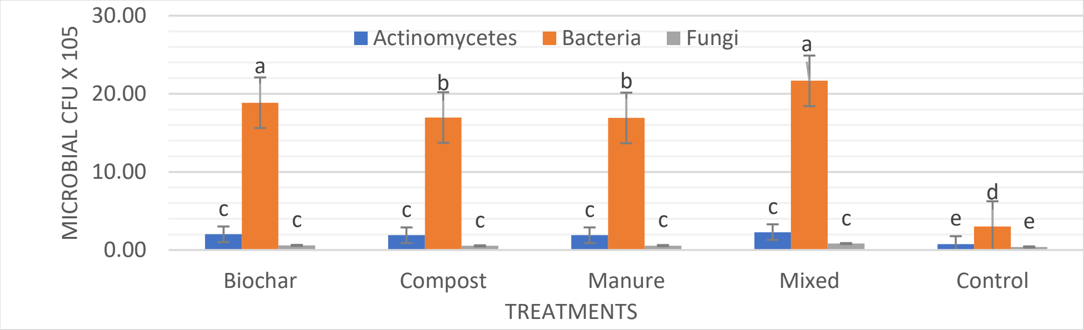


Figure 7.2. The abundance (CFU x 10^5) of actinomycetes, bacteria and fungi at each amended plot. Values represent means ± SD (n = 3).

Different letters indicate significant differences among soil amendments ($p \leq 0.05$) using Duncan's multiple comparison test. Mixed = biochar, compost, manure and bacterial inoculation

7.3.1.2. Actinomycetes Populations in Organic Amended Soil

This study showed that the number of actinomycetes population was significantly affected by organic amendments compared to the control. The mean actinomycetes counts were between 2.10 x 10^6 – 1.02 x 10^6 g^{-1} dry soils in amended plots compared to 6.3 x 10^5 to 1.58 x10^6 g^{-1}dry soil of the control plot. There was an inconsistent monthly variation in the actinomycetes population (Figure 7.3). The higher actinomycetes population count was recorded in April (wet season); whereas the lowest count was observed in December (dry season) (Figure 7.3).

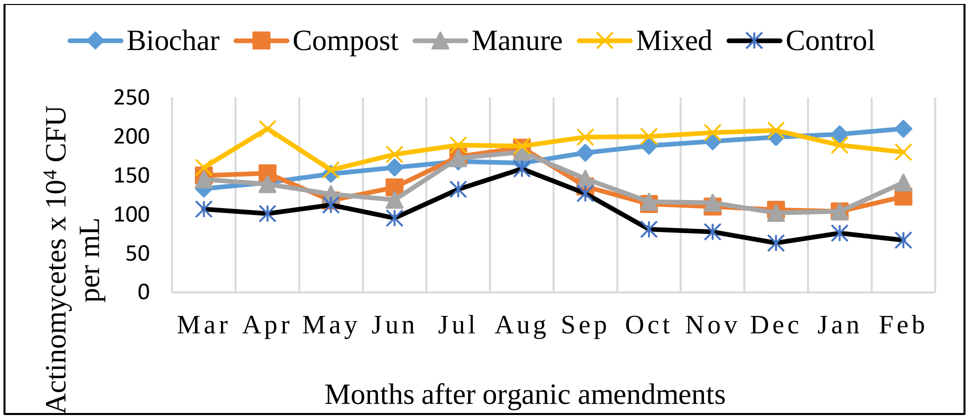


Figure 7.3. Actinomycetes counts after different organic amendments for consecutive 12 months and values represent means (n = 3). Mixed = biochar + compost + manure

7.3.1.3. Bacterial Populations in Organic Amended Soil

This study revealed that the bacterial loads were significantly affected by the application of organic amendments compared to the control (Figure 7.4). Plots with organic amendments showed 2.13 x 10^7 – 1.12 x 10^7 g^{-1} dry soil, whereas 1.76 x 10^7 -5.8 x 10^6 g^{-1} dry soil was recorded from nonamended plots. A similar pattern was also noted for the bacterial population as observed in the case of actinomycetes count with biochar and mixed treated plots (Figure7.4). The maximum bacterial counts were recorded in July (wet season) and the lowest were counted in November (Dry season) (Figure 7.4).

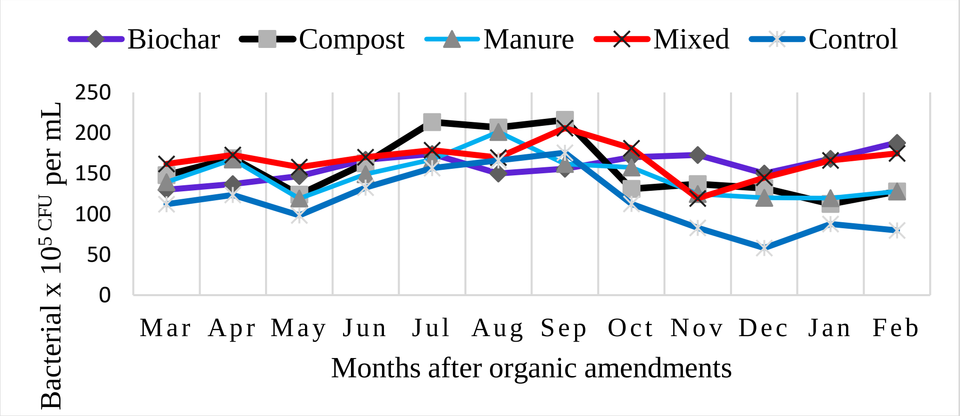


Figure 7.4. Bacterial counts after different organic amendments for consecutive 12 months and values represent mean (n = 3). Mixed = biochar + compost + manure

7.3.1.4. Fungal Populations in Organic Amended Soil

In this study, increase in fungal counts was obtained from organically amended plots compared to the control (Figure 7.5). The highest fungal count of 8.2 x 10^4 g^{-1} dry soil was recorded from mixed amended soil, while the least number was recorded from the control plot (2.4 x 10^4 g^{-1}) dry soil. There were inconsistent monthly counts of the fungal population. As compared to actinomycetes and bacterial abundance, the fungal counts were very low. The highest fungal load was observed in June (wet season) while the lowest count was noted in October (dry season) (Figure 7.5).

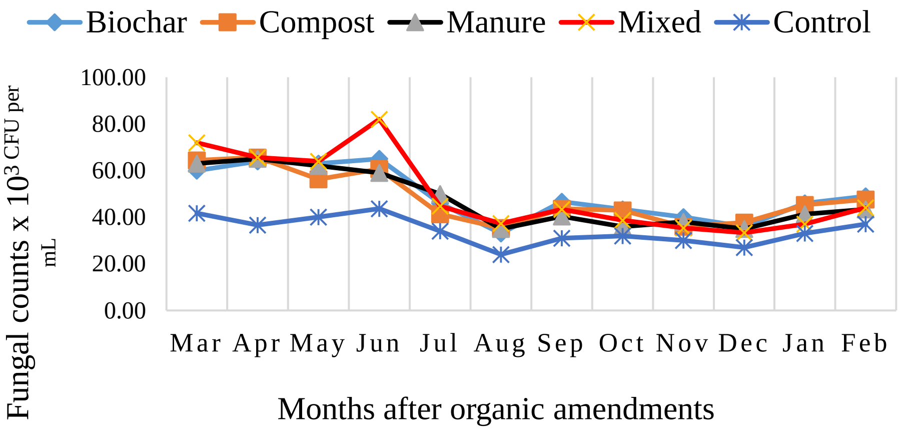


Figure 7.5. Fungal population after different organic amendments for consecutive 12 months and values represent means (n = 3). Mixed = biochar + compost + manure

7.3.2. Multipurpose Trees Establishment

7.3.2.1. Plant Growth Parameters

The growth of multipurpose trees amended with biochar, compost, manure, inoculated with bacteria and their combinations were measured every three months and the result showed a significant increase in height, branch numbers, and girth of the tree species biomass to the control (Table 7.2). Change in height was one of the clear indicators of the plants in the field and all the tree species showed significantly ($p < 0.05$) higher stem height on plots treated with OAs and BI compared to the control (Table 7.2). The results indicated that soil enrichment generally increased the growth of tree species on amended soils. An integrated treatment of OAs + BI displayed the best growth effect on the stem height (1, 0.42 and 0.39 m/plant) in apple, acacia, and prunus tree species respectively compared to the control (Table 7.2). The maximum increase in stem height of all the trees was observed from BI x OAs treatment. The trees grew in height (1 m in apple and 40 cm in acacia and 44 cm in prunus). Inoculation with bacteria significantly enhanced tree height (97.25, 34.25, 38.75 cm/plant) in apple, acacia, and prunus trees, respectively. Although their height steadily increased with time, the growth rate was small: 55 cm/year, 29.5 cm/year, and 21 cm/year for apple, acacia and prunus, respectively (Table 7.2).

Table 7.2. Measurements of stem height (SH) (mean ± standard deviation) taken within three months intervals at different soil treatments.

Tree species	Treatments					
	BAS	CAS	MAS	BI	BI x OAs	CTRL
Apple (SH in cm from 3-12 months)						
3	64.50 ±3.1^{ab}	67.50±4.2^{ab}	66.25±2.6^{ab}	75.75±4.9^{a}	80.75 ±3.1^{a}	47.00±1.5^{c}
6	72.75±5.3^{ab}	69.50±5.3^{ab}	69.25±2.8^{ab}	78.25±8.1^{a}	84.75±4.0^{a}	50.50±4.9^{c}
9	75.50±4.9^{ab}	71.50±7.0^{ab}	71.00±3.2^{ab}	79.50±1.3^{a}	87.75±3.9^{a}	51.50±3.1^{c}
12	90.75±8.5^{a}	73.75±6.7^{ab}	73.00±3.2^{ab}	97.25±2.5^{a}	1.00±4.0^{a}	54.25±4.9^{c}
Acacia (SH in cm from 3-12 months)						
3	18.25±1.7^{bc}	20.25±2.2^{b}	20.50±2.1^{b}	21.75±3.9^{b}	28.75±2.5^{a}	14.25±0.6^{c}
6	21.50±4.2^{b}	23.25±1.7^{b}	22.00±2.6^{b}	25.50±2.1^{ab}	31.50±3.1^{a}	14.75±1.0^{c}
9	21.75±2.5^{b}	29.00±2.2^{a}	27.75±1.7^{ab}	30.50±2.1^{a}	33.50±3.1^{a}	15.25±1.0^{c}
12	27.25±2.5^{b}	31.00±2.6^{b}	33.00±2.9^{ab}	34.25±1.7^{ab}	42.50±1.3^{a}	16.25±a.5^{c}
Prunus (SH in cm from 3-12 months)						
3	25.7±2.6^{abc}	26.0±2.6^{abc}	24.50±5.3^{bc}	31.75±2.6^{ab}	33.50±1.3^{a}	19.00±1.5^{c}
6	28.75±3.6^{ab}	29.25±4.4^{ab}	29.50±2.1^{ab}	33.50±2.6^{a}	35.50±1.3^{a}	21.00±2.0^{b}
9	33.75±3.3^{a}	31.25±2.5^{ab}	33.75±3.0^{a}	35.25±2.6^{a}	37.00±2.2^{a}	22.50±1.0^{b}
12	36.25±3.0^{a}	38.75±3.3^{a}	36.25±2.9^{a}	38.75±3.4^{a}	39.00±3.6^{a}	23.75±1.5^{b}

BAS =biochar amended soil, CAS=compost amended soil, MAS=manure amended soil, PGPR= plant growth-promoting rhizobacteria, BI x OAs = BAS +CAS+MAS+ bacteria, CTRL=control. Numbers in each row followed by the same lower-case letters within the row do not differ significantly at $p < 0.05$ according to the Duncan test.

The largest girths of the plants were observed in plots treated with OAs and BI compared to the control (Table 7.3). Integrated treatment of OAs x BI showed a significant ($p \leq 0.05$) growth effect on the stem diameter of the tree species compared to the control (Table 7.3). The stem diameters were also steadily increased with time, within the three months intervals: 9.5 cm/year, 1.98 cm/year, and 9 cm/year for apple, acacia and prunus, respectively compared to the control.

Table 7.3. Measurements of stem or trunk diameter (SD) (mean ± standard deviation) taken within three months intervals at different soil treatments.

Tree species	Treatments					
	BAS	CAS	MAS	BI	BI x OAs	CTRL
Apple (SD in cm from 3-12 months)						
3	5.50 ± 1.3^{a}	4.5 ± 1.3^{ab}	5.50 ± 1.3^{a}	6.00 ± 1.6^{a}	7.00 ± 10.8^{a}	2.5 ± 2.5^{b}
6	7.00 ± 1.8^{ab}	4.75 ± 1.3^{bc}	6.00 ± 0.8^{ab}	7.00 ± 0.8^{ab}	8.00 ± 0.8^{a}	2.75 ± 2.1^{c}
9	7.50 ± 2.1^{abc}	5.25 ± 1.0^{cd}	6.50 ± 0.6^{bc}	8.25 ± 1.0^{ab}	10.00 ± 2.2^{a}	3.00 ± 2.0^{d}
12	8.00 ± 1.6^{b}	6.00 ± 1.2^{b}	7.00 ± 0.8^{b}	10.50 ± 1.3^{a}	12.00 ± 1.6^{a}	3.25 ± 1.5^{c}
Acacia (SD in cm from 3-12 months)						
3	0.58 ± 0.1^{bc}	1.25 ± 0.8^{a}	0.78 ± 0.2^{abc}	0.93 ± 0.2^{abc}	1.13 ± 0.2^{ab}	0.30 ± 0.1^{c}
6	0.75 ± 0.2^{bc}	1.43 ± 0.8^{a}	0.90 ± 0.2^{abc}	1.15 ± 0.2^{ab}	1.40 ± 0.2^{a}	0.38 ± 0.1^{c}
9	0.90 ± 0.3^{bc}	1.53 ± 0.7^{ab}	1.15 ± 0.2^{ab}	1.35 ± 0.2^{ab}	1.78 ± 0.2^{a}	0.50 ± 0.2^{c}
12	1.05 ± 0.4^{cd}	1.83 ± 0.7^{ab}	1.40 ± 0.1^{bc}	1.90 ± 0.3^{ab}	2.28 ± 0.3^{a}	0.63 ± 0.2^{d}
Prunus (SD in cm from 3-12 months)						
3	4.68 ± 2.6^{bc}	6.00 ± 3.1^{b}	4.33 ± 2.2^{bc}	6.33 ± 3.4^{ab}	8.33 ± 4.3^{a}	3.33 ± 0.6^{c}
6	6.00 ± 3.1^{bc}	7.00 ± 3.6^{b}	6.00 ± 3.1^{bc}	7.67 ± 3.9^{b}	9.33 ± 4.8^{a}	4.33 ± 0.6^{c}
9	6.33 ± 3.3^{cd}	8.00 ± 4.1^{bc}	7.00 ± 3.6^{b}	9.33 ± 4.8^{ab}	10.67 ± 5.6^{a}	4.67 ± 0.6^{d}
12	10.00 ± 5.3^{bc}	9.00 ± 4.6^{bc}	8.33 ± 4.3^{c}	11.33 ± 5.8^{ab}	11.33 ± 4.8^{a}	5.68 ± 0.6^{d}

BAS =biochar amended soil, CAS=compost amended soil, MAS=manure amended soil, PGPR= plant growth-promoting rhizobacteria, BI x OAs = BAS + CAS + MAS + bacteria, CTRL= control. Numbers in each row followed by the same lower-case letters within the row do not differ significantly at $p < 0.05$ according to the Duncan test.

Similarly, the number of branches produced by each species showed a significant difference between amended and inoculated plots and the control (Table 7.4). The highest branch numbers were observed in plots treated with OAs and BI compared to the control ($p < 0.05$). Similarly, OAs x BI significantly ($p \leq 0.05$) displayed the highest number of branches compared to the control (Table 7.4). Generally, in all plant growth parameters, soil enrichment with OAs and microbial application greatly increased the growth of tree species (Table 7.4). The number of branches steadily increased with time i.e., 4.5 cm/year, 3.75 cm/year, and 3.55 cm/year for apple, acacia, and prunus, respectively compared to the control plots.

Table 7.4. Measurements of the number of branches (NB) (mean ± standard deviation) taken within three months intervals at different soil treatments.

Tree species	Treatments					
	BAS	CAS	MAS	BI	BI x OAs	CTRL
Apple (NB within 3-12 months)						
3	2.00 ± 1.2^{bc}	1.25 ± 0.5^{ab}	1.00 ± 0.8^{bc}	2.00 ± 0.8^{ab}	2.5 ± 0.6^{a}	0.50 ± 0.6^{c}
6	2.50 ± 1.3^{a}	2.25 ± 1.0^{ab}	1.50 ± 0.6^{ab}	2.25 ± 1.0^{ab}	3.00 ± 0.8^{a}	0.75 ± 0.0^{c}
9	3.25 ± 1.0^{ab}	2.75 ± 0.5^{ab}	2.25 ± 0.5^{b}	3.50 ± 0.6^{b}	3.75 ± 0.5^{a}	1.25 ± 0.6^{c}
12	4.00 ± 0.8^{ab}	3.25 ± 0.5^{b}	3.50 ± 0.6^{b}	4.25 ± 1.0^{ab}	5.00 ± 0.8^{a}	1.26 ± 0.6^{c}
Acacia (NB within 3-12 months)						
3	0.50 ± 0.6^{ab}	1.25 ± 0.5^{b}	1.00 ± 0.8^{ab}	1.75 ± 1.0^{a}	2.00 ± 0.8^{a}	0.25 ± 0.6^{c}
6	1.25 ± 1.0^{ab}	2.00 ± 0.8^{a}	1.50 ± 0.6^{ab}	2.25 ± 0.5^{a}	2.25 ± 1.3^{a}	0.50 ± 00.6^{c}
9	1.75 ± 1.0^{bc}	2.50 ± 0.6^{ab}	2.25 ± 0.5^{b}	3.250 ± 0.6^{a}	3.50 ± 0.6^{a}	0.75 ± 1.0^{c}
12	2.50 ± 0.6^{b}	3.00 ± 0.0^{b}	3.00 ± 0.8^{b}	3.50 ± 1.0^{a}	4.00 ± 0.8^{a}	1.25 ± 0.6^{c}
Prunus (NB within 3-12 months)						
3	1.75 ± 1.0^{abc}	1.75 ± 0.5^{abc}	1.25 ± 1.0^{bc}	2.50 ± 0.6^{ab}	3.00 ± 0.8^{a}	0.75 ± 1.0^{d}
6	2.50 ± 0.6^{bc}	2.50 ± 0.6^{bc}	2.00 ± 0.8^{c}	3.00 ± 0.8^{ab}	3.50 ± 0.6^{a}	1.00 ± 0.6^{d}
9	2.75 ± 0.5^{b}	3.50 ± 0.6^{ab}	3.00 ± 0.8^{b}	3.75 ± 0.5^{ab}	4.25 ± 0.5^{a}	1.50 ± 1.0^{c}
12	3.50 ± 0.6^{b}	4.25 ± 0.5^{ab}	3.75 ± 1.0^{ab}	4.25 ± 0.5^{ab}	4.30 ± 0.8^{a}	2.00 ± 0.6^{c}

BAS =biochar amended soil, CAS=compost amended soil, MAS=manure amended soil, PGPR= plant growth promoting rhizobacteria, BI x OAs = BAS + CAS + MAS + bacteria, CTRL=control. Numbers in each row followed by the same lower-case letters within row do not differ significantly at $p < 0.05$ according to Duncan test.

7.3.2.2. Survival rate of tree species

At the end of one year after planting, the apple tree indicated the highest survival rate followed by acacia and prunus (Figure 7.6). Without considering the types of treatments confirmed that apple, acacia, and prunus trees exhibited 80%, 66.67%, and 51.67% survival rates, respectively (Figure 7.6). The result shows that the apple tree is the best species among the other tested species for growing on degraded soil after OAs and bacterial inoculations.

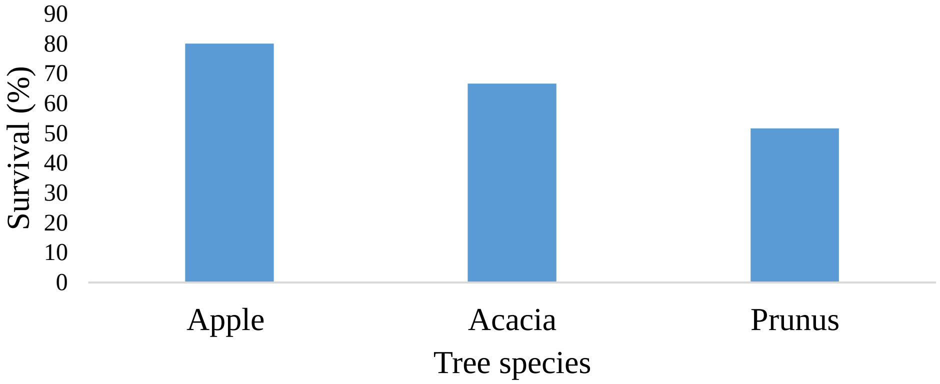


Figure 7.6. The total survival of transplanted plant species in OA soil treatments after 0ne year growing seasons at North Shewa Oromia National Regional State, Ethiopia.

Organic amendments such as biochar, compost, manure, and bacterial inoculations singly or in combination showed 40 to 90% increases in the survival rate of the selected trees compared to the 30-50% survival rate recorded from the control plots at the end of a year after transplantation (Figure 7.7). The survival rate showed a pattern of BI x OAs > bacteria > BAS > CAS > MAS > CTRL, respectively for all the tree species studied.

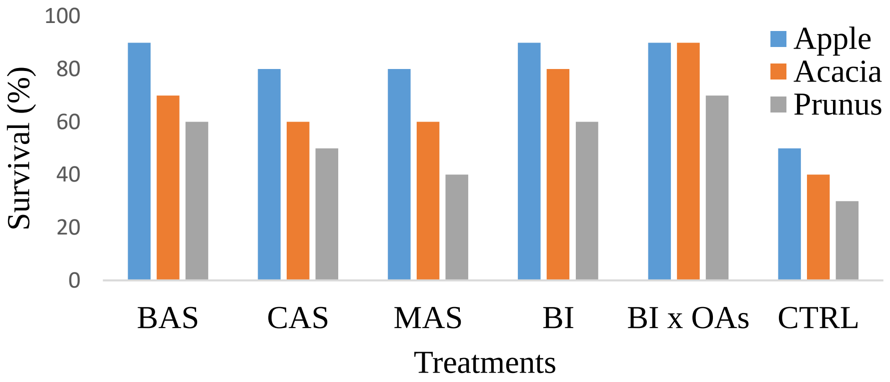


Figure 7.7. Treatments wise comparisons of survival rate of species in OA soil treatments at North Shewa Oromia National Regional State, Ethiopia.

BAS = biochar amended soil, CAS=compost amended soil, MAS=manure amended soil, BI x OAs = BAS +CAS + MAS + bacteria, CTRL= control

7.4. Discussion

This study revealed that soil microbial abundance (counts) generally increased following the application of OAs compared to the control plot. The availability of nutrients in organically amended plots resulted in the maximum microbial population. This might be because of the presence of sugars and amino acids in OAs that contribute to microbiological activity and fertility and elevated levels of enzymes secreted by soil microbes (Ali *et al.*, 2019; Scotti *et al.*, 2015). In this study, bacterial dominance was shown followed by actinomycetes. The variation in microbial abundance is that OAs incorporated into the soil tend to decompose faster and stimulate bacterial activity, whereas those left on the soil surface decompose at a slower rate and tend to stimulate actinomycetes and fungal activity in the soil (Lori *et al.*, 2017).

The highest microbial counts were obtained in a combined treatment indicating the presence of a synergistic role among treatments. Likewise, different amendments have different impacts on the microbial activities in the soil as verified elsewhere with the general assumption that organic fertilization increases microbial abundance (Chaudhry *et al.*, 2012). Sabet *et al.* (2013) reported that the counts of bacteria, actinomycetes, and fungi from compost amended soil ranged from 8.2×10^7 to 21.6×10^7 CFU/g, 0.4×10^5 to 2.0×10^5 CFU/g, and 5.6×10^4 to 51.4×10^4 CFU/g, respectively. According to Nakhro and Dkhar (2010), farmyard manure treated fields increased bacterial and fungal population counts between 2.2×10^6 CFU/g and 4.5×10^3 CFU/g, respectively compared to the control plot. Furthermore, other researchers have shown that incorporation of OAs increased soil microbial activity Lukac *et al.* (2017), microbial diversity (Girvan *et al.*, 2004; Kirk *et al.*, 2004), and microbial density (Van Bruggen and Semenov, 2000). Application of organic C and clay content influence microbial functional diversity by 61.8–82.8% in particular at the end of the 12th month (Li *et al.*, 2018).

The other significant change observed in the microbial counts during the experimental period was the inconsistent microbial count across each sampling month (Figures 7.3, 7.4, 7.5). This is mainly associated with soil moisture, which is one of the major factors that can change the microbial community structure and activity (Classen *et al.*, 2015). In this study, the highest microbial counts were recorded during the wet seasons (June, July, and April) while the lowest counts were observed during dry seasons (October, November, and December). The reason is that soil moisture is never stable in the natural environment. Bacteria, fungi and actinomycetes were the most abundant during the wet season (Keya *et al.*, 1982; Wang *et al.*, 2016). The possible reasons for these variations might be due to, in the wet season, the ample moisture and suitable temperature are favorable for the growth of soil microorganisms (Lixia *et al.*, 2004).

In the present study, there was also a significant ($p \leq 0.05$) difference observed in compost and manure amended plots compared to the control. The data showed an increase in bacterial and actinomycetes counts in biochar amended soil (BAS) except in the case of the fungal population (Figure 7.2). The maximum microbial counts in BAS are attributed to its natural characteristics to release nutrients slower compared to the others. There is a notable fluctuation in fungal counts in biochar amended soil. The possible reason for this variation could be the antagonism among the microflora present in the biochar (Rutigliano *et al.*, 2014). Biochar provides shelter for soil microbes with pore structures and surfaces and modifies microbial habitats by improving soil properties (Quilliam *et al.*, 2013). Palansooriya *et al.* (2019) have shown that biochar application as a soil amendment can increase microbial population and activities. The other possible reasons responsible for the increase in microbial abundance is the higher availability of nutrients or labile organic matter on biochar surface Bruun *et al.* (2012), and less competition (Lehmann *et al.*, 2011). The microbial reproduction rate has also been shown to increase in some biochar-amended soils (Steiner *et al.*, 2009).

The development of natural populations of soil microorganisms was reported in soils amended with urban refuse del Mar Alguacil *et al.* (2009) and sugar beet residue (Caravaca *et al.*, 2005). Gennari *et al.* (2007) reported that the number of actinomycetes was 10^5 CFU g^{-1} soil in every soil studied. However, pH and moisture content could affect the actinomycetes population among the different OAs. According to Parham *et al.* (2003), cattle manure application promoted the growth of bacteria, but not fungi when compared to the control soil. This was partially attributed to limitations of pH values (the higher pH value may pose fungal growth) in the manure treated soils. Changes in soil microbial communities (e.g., bacteria, actinomycetes) were more noticeable in soils subjected to organic manure applications than in the control soils (Dong *et al.*, 2014). In general, the application of different OAs helps the native microbes in soil by improving soil aeration, water holding capacity and stimulates microorganisms in the soil that make plant nutrients readily available leading to higher yield and better quality of plants.

In this field experiment, application of OAs and inoculation with bacteria added separately or OAs x BI, were very efficient to promote the stem growth, diameter, and branch numbers in multipurpose trees tested (Tables 7.2, 7.3, 7.4). This could be due to the inoculation of bacteria that serve mainly as a booster for plant growth during its initial life stages and a synergistic effect may occur. Moreover, other soil microbes such as native rhizobia may interact with the legume trees. Also, inoculants can play an essential role in nutrient uptake and therefore enhance the biomass promotion in plants (Souza *et al.*, 2015).

Similarly, a survival rate of 60-90%, was reported on degraded desert soil after microbial inoculation and compost amendment (Bashan *et al.*, 2012). Moreover, the average survival rate of all inoculated three cactus species after 3.5 years was 76% as reported in desert soil compared to the control (2%) (Bashan *et al.*, 2000). Similarly, Bashan *et al.* (2000) reported mentioned the survival rates of mesquite amargo (*Prosopis articulata*) and yellow palo verde (*Parkinsonia microphylla*) were over 80% and the survival rate of mesquite was almost 100% after 10 months of cultivation with inoculations of *Azospirillum brasilense* and *Bacillus pumilus*, *Glomus* sp. and supplementation with compost (Bashan *et al.*, 2009).

Native desert trees respond to inoculation with growth-promoting microorganisms in a manner resembling crops and cacti (Bashan and De-Bashan, 2005). Inoculation of beneficial microbes to degraded lands may improve *Jatropha curcas* by enhancing plant resistance to adverse environmental stresses (water and nutrient deficiency and heavy metal contamination) (Kumar *et al.*, 2015). Thomas and Gale (2015) have also reported a mean biomass growth response of 41% for woody plants after biochar amendment. Compost is another soil amendment that increased plant growth (Soumare *et al.*, 2003). It has been repeatedly suggested that the combination of biochar with compost may be a promising strategy to promote plant growth and performance, having positive synergistic effects on soil properties and plant growth responses (plant height and root biomass) (Liu *et al.*, 2012; Steiner *et al.*, 2010). Synergistic effects of a biochar-compost blend on plant growth and performance are thought to be mediated by sorption of nutrients by the porous biochar matrix (Steiner *et al.*, 2010), stimulation of microbial colonization (Pietikäinen *et al.*, 2000), degradation of possible noxious pyrogenic substances (Tuomela *et al.*, 2000), improvement of the biochar surface reactivity through accelerated oxidative aging (Cheng and Lehmann, 2009; Zimmerman, 2010) and dissolved organic carbon sorption (Prost *et al.*, 2013). Bashan *et al.* (2012) also indicated that, native leguminous trees (mesquite amargo (*Prosopis articulate*), yellow palo verde (*Parkinsonia microphylla*) and blue palo verde (*Parkinsonia florida*) responded well (height, number of branches, and diameter of the main stem). This is achieved following the inoculation with PGPB, AM fungi, and compost supplementation after three months of application. Generally, among the treatments, OAs x BI treatments and PGPR revealed their effectiveness in the field by promoting higher survival rate, high stem height, and girth diameter and branch number of the tree species. This might be due to the presence of microbial inoculants that could have helped in mitigating drought, protecting plants from soil-borne pathogens and increased mineral availability.

7.5. Conclusion

From this study, it can be concluded that OAs particularly biochar, compost, manure, and their integrated application showed a better impact on the microbial population compared to the control plots. The higher microbial abundance was recorded in the plot with the mixed application. The amendment of degraded soils with OAs and BI improved plant performance under degraded soil conditions. By and large, the application of combined treatments (BI and OAs) seems to be the most appropriate method to aid in the restoration of both plant cover and soil quality in degraded soil.

Chapter 8

8. Conclusion and recommendation

Based on the results of this work, the following conclusions and recommendations are forwarded.

8.1. conclusion

- The application of organic amendments (OAs) (biochar, compost, manure and their combination) significantly enhanced soil fertility.
- Mixed application of OA showed a remarkable increase in soil fertility status and the performance of cover crops.
- The rhizosphere of woody plant species from degraded soil harbor genetically and physiologically diverse plant growth promoting bacteria (with 11 different genera).
- *Pseudomonas* and *Ochrobactrum* species are the most dominant while *Bacillus* species are the most diverse groups.
- The rhizobacterial species of *P. fluorescent*, *P. putida*, *P. polymyxa,* and *A. calcoaceticus* could increase soil fertility.
- Rhizobacterial isolates from degraded soil showed plant growth promoting traits in vitro, under greenhouse and field conditions.
- The potentials of PGPR in degraded land rehabilitation is proved to be practical and eco-friendly technologies to implemented.
- The establishment of multipurpose trees on degraded land with the application of OAs and rhizobacterial inoculation were successful with a survival rate of 52-80%.
- The selection of the most efficient rhizobacterial strains and their combined effect with organic inputs seems to be a critical point that drives the effectiveness of using these biotechnological tools for the revegetation and rehabilitation of degraded soils.

8.2. Recommendations

- ✓ The application of different types of organic amendments will be suggested as a toolbox to transform marginal and abandoned land into productive land.
- ✓ A reliable strategy is needed to combine and communicate the available tools so that environmental services is maintained or even increased.
- ✓ The role of potential and indigenous microorganisms in the restoration of degraded soil will be considered for sustainable land management.
- ✓ Land-owners should be educated and aware in sustainable land use, agribusiness, marginal lands will be unlocked and strengthened, and local stakeholders will defend their region from further degradation to establish economically sound soil/land management systems.
- ✓ Large scale rehabilitation of many fragile and degraded areas will be needed using eco-friendly bacterial application and organic inputs.
- ✓ The rhizobacterial species of *P. fluorescent*, *P. putida*, *P. polymyxa,* and *A. calcoaceticus* are suggested for further large-scale rehabilitation.
- ✓ To this end, the finite nature of soil resources must never be taken for granted, they must be used, improved, and restored.

Future Research Directions

- Effective prevention of soil degradation requires both local management and macro policy approaches that promote sustainability of ecosystem services. It is advisable to focus on prevention, because attempts to rehabilitate degraded areas are costly and tend to deliver limited results.
- Impacts of new technologies on land degradation and their social and economic ramifications need more research.
- Checklist or key could be developed to facilitate identification of factors that are important for determining the most appropriate amendments and practices.
- Application rates of OAs could be determined experimentally to establish a feasible restoration protocol under a variety of restoration scenarios.
- There is a need to devise a strategy that properly handle and dispose solid wastes generated by diverse sources in Ethiopia.
- Further research into the integration of these amendments into severely degraded landscapes during restoration projects needs to be conducted and can reduce long-term maintenance costs and create a foundation for sustainable above- and belowground communities.

9. References

Abbasi, M., Hina, M., Khalique, A. and Razaq Khan, S. (2007). Mineralization of three organic manures used as nitrogen source in a soil incubated under laboratory conditions. *Commun. Soil. Sci. Plant. Anal.,* **38**: 1691-1711.

Abebe Nigussie, Endalkachew Kissi, Mastawesha Misganaw and Gebermedihin Ambaw. (2012). Effect of biochar application on soil properties and nutrient uptake of lettuces (Lactuca sativa) grown in chromium polluted soils. *Am Eurasian J Agric Environ Sci.,* **12**: 369-376.

Abhilash, P., Tripathi, V., Edrisi, S.A., Dubey, R.K., Bakshi, M., Dubey, P.K., Singh, H. and Ebbs, S.D. (2016). Sustainability of crop production from polluted lands. *Energy, Ecology and Environment,* **1**: 54-65.

Acosta-Martinez, V., Mikha, M.M., Sistani, K.R., Stahlman, P.W., Benjamin, J.G., Vigil, M.F. and Erickson, R. (2011). Multi-location study of soil enzyme activities as affected by types and rates of manure application and tillage practices. *Agri.,* **1**: 4-21.

Ahemad, M. and Khan, M.S. (2012). Effect of fungicides on plant growth promoting activities of phosphate solubilizing Pseudomonasputida isolated from mustard (Brassica compestris) rhizosphere. *Chemosphere,* **86**: 945-950.

Ahemad, M. and Mulugeta Kibret. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J. King Saud University-Sci.,* **26**: 1-20.

Ahmad, F., Ahmad, I. and Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.,* **163**: 173-181.

Ahmad, M., Zahir, Z.A., Khalid, M., Nazli, F. and Arshad, M. (2013). Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol. Biochem.,* **63**: 170-176.

Alebachew Gebrie. (2015). Evaluating the sustainability of communal land rehabilitation practices as a disaster risk reduction strategy and adaptation measures to climate change: a case study from Legambo District, Northern Ethiopia. Type of thesis University of South Africa, Addis Ababa, pp. 1-117.

Alemayehu Getahun, Diriba Muleta, Fassil Assefa and Solomon Kiros. (2020a). Plant Growth-Promoting Rhizobacteria Isolated from Degraded Habitat Enhance Drought Tolerance of Acacia (Acacia abyssinica Hochst. ex Benth.) Seedlings. *Int. J. Microbiol.,* **2020**: 1-13.

Alemayehu Getahun, Diriba Muleta, Fassil Assefa, Solomon Kiros and Hungria, M. (2020b). Biochar and Other Organic Amendments Improve the Physicochemical Properties of Soil in Highly Degraded Habitat. *Eur. J. Eng. Res. Sci.,* **5**: 331-338.

Alemayehu Getahun, Solomon Kiros, Diriba Muleta and Fassil Assefa. (2020c). Genetic and metabolic diversities of rhizobacteria isolated from degraded soil of Ethiopia. *Heliyon,* **6**: 1-10.

Alguacil, M., Torrecillas, E., Caravaca, F., Fernández, D., Azcón, R. and Roldán, A. (2011). The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. *Soil. Biol. Biochem.,* **43**: 1498-1508.

Ali, S., Rawat, L., Meghvansi, M. and Mahna, S. (2009). Selection of stress-tolerant rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India. *J. Agri. Biol. Sci.,* **4**: 13-18.

Ali, S.S. and Vidhale, N. (2013). Bacterial siderophore and their application: a review. *Int. J. Curr. Microbiol. Appl. Sci.,* **2**: 303-312.

Ali, W., Nadeem, M., Ashiq, W., Zaeem, M., Gilani, S.S.M., Rajabi-Khamseh, S., Pham, T.H., Kavanagh, V., Thomas, R. and Cheema, M. (2019). The effects of organic and inorganic phosphorus amendments on the biochemical attributes and active microbial population of agriculture podzols following silage corn cultivation in boreal climate. *Sci. Rep.,* **9**: 1-17.

Alikhani, H. and Mohamadi, L. (2010). Assessing tolerance of rhizobial lentil symbiosis isolates to salinity and drought in dry land farming condition. **In:** *Proceeding Assessing tolerance of rhizobial lentil symbiosis isolates to salinity and drought in dry land farming condition*, pp. 1-6.

Almaghrabi, O.A., Massoud, S.I. and Abdelmoneim, T.S. (2013). Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi J. Biol. Sci.,* **20**: 57-61.

Altieri, M.A. and Toledo, V.M. (2011). The agroecological revolution in Latin America: rescuing nature, ensuring food sovereignty and empowering peasants. *J. Peasant Stud.,* **38**: 587-612.

Aly, M.M., El Sayed, H. and Jastaniah, S.D. (2012). Synergistic effect between Azotobacter vinelandii and Streptomyces sp. isolated from saline soil on seed germination and growth of wheat plant. *J Am Sci,* **8**: 667-676.

Amare Haileslassie, Priess, J., Veldkamp, E., Demil Teketay and Lesschen, J.P. (2005). Assessment of soil nutrient depletion and its spatial variability on smallholders' mixed farming systems in Ethiopia using partial versus full nutrient balances. *Agri. Ecosy. Environ.,* **108**: 1-16.

Amarger, N., Macheret, V. and Laguerre, G. (1997). Rhizobium gallicum sp. nov. and Rhizobium giardinii sp. nov., from Phaseolus vulgaris nodules. *Int. J. Syst. Evol. Microbiol.,* **47**: 996-1006.

An, S., Couteau, C., Luo, F., Neveu, J. and DuBow, M.S. (2013). Bacterial diversity of surface sand samples from the Gobi and Taklamaken deserts. *Microb. Ecol.,* **66**: 850-860.

Anawar, H.M., Akter, F., Solaiman, Z.M. and Strezov, V. (2015). Biochar: an emerging panacea for remediation of soil contaminants from mining, industry and sewage wastes. *Pedosphere,* **25**: 654-665.

Anjum, S.A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M.F. and Ali, I. (2017). Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. *Front. Plant Sci.,* **8**: 1-9.

Ansari, F.A. and Ahmad, I. (2018). Biofilm development, plant growth promoting traits and rhizosphere colonization by Pseudomonas entomophila FAP1: A Promising PGPR. *Adv. Microbiol,* **8**: 235.

Armada, E., Barea, J.-M., Castillo, P., Roldán, A. and Azcón, R. (2015). Characterization and management of autochthonous bacterial strains from semiarid soils of Spain and their interactions with fermented agrowastes to improve drought tolerance in native shrub species. *Appl Soil Ecol,* **96**: 306-318.

Armada, E., Portela, G., Roldán, A. and Azcón, R. (2014). Combined use of beneficial soil microorganism and agrowaste residue to cope with plant water limitation under semiarid conditions. *Geoderma,* **232**: 640-648.

Asari, S.Y. (2015). ***Studies on plant-microbe interaction to improve stress tolerance in plants for sustainable agriculture***. Acta Universitatis agriculturae Sueciae pp. 1652-6880.

Ashish, T., Shikha, D., Kumar, S.N. and Shivesh, S. (2016). Isolation, screening and characterization of PGPR isolated from rhizospheric soils of Pigeonpea. *Res. J. Biot.* , **11**: 3.

Assel, P. (2006). Evaluating the usefulness of Acacia auriculiformis in ameliorating surface mine degraded lands. *Unpublished B. Sc. dissertation, Department of Agroforestry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*: 1-24.

Aulakh, M.S. and Sidhu, G.S. (2015). Soil degradation in India: Causes, major threats, and management options. **In:** *Proceeding Soil degradation in India: Causes, major threats, and management options*, pp. 151-156.

Aune, J.B., Bussa, M.T., Asfaw, F.G. and Ayele, A.A. (2001). The ox ploughing system in Ethiopia: can it be sustained? *Outlook Agric,* **30**: 275-280.

Azcón, R., del Carmen Perálvarez, M., Roldán, A. and Barea, J.-M. (2010). Arbuscular mycorrhizal fungi, Bacillus cereus, and Candida parapsilosis from a multicontaminated soil alleviate metal toxicity in plants. *Microb. Ecol.,* **59**: 668-677.

Bainbridge, D.A. (2012). ***A guide for desert and dryland restoration: new hope for arid lands***. Island Press, Washington, USA, pp. 26 - 416

Banerjee, S., Palit, R., Sengupta, C. and Standing, D. (2010). Stress induced phosphate solubilization by'Arthrobacter'Sp. And'Bacillus' sp. isolated from tomato rhizosphere. *Aust. J. Crop. Sci.,* **4**: 378.

Bano, A. and Fatima, M. (2009). Salt tolerance in Zea mays (L). following inoculation with Rhizobium and Pseudomonas. *Biol. Fertility Soils,* **45**: 405-413.

Barea, J., Palenzuela, J., Cornejo, P., Sánchez-Castro, I., Navarro-Fernández, C., Lopéz-García, A., Estrada, B., Azcón, R., Ferrol, N. and Azcón-Aguilar, C. (2011). Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. *J. Arid Environ.,* **75**: 1292-1301.

Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S. and Kalra, A. (2014). ACC deaminase-containing Arthrobacter protophormiae induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in Pisum sativum. *J. Plant. Physiol.,* **171**: 884-894.

Barrow, C. (2012). Biochar: potential for countering land degradation and for improving agriculture. *Appl. Geogr.,* **34**: 21-28.

Bashan, Y. (1998). Inoculants of plant growth-promoting bacteria for use in agriculture. *Biot. Adv.,* **16**: 729-770.

Bashan, Y., Davis, E.A., Carrillo-Garcia, A. and Linderman, R.G. (2000). Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran Desert. *Appl. Soil. Ecol.,* **14**: 165-175.

Bashan, Y. and De-Bashan, L. (2005). Plant growth-promoting. *Ency. Soils. Environ,* **1**: 103-115.

Bashan, Y., de-Bashan, L.E., Prabhu, S. and Hernandez, J.-P. (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil,* **378**: 1-33.

Bashan, Y., Kamnev, A.A. and de-Bashan, L.E. (2013). Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. *Biol. Fertil. Soils.,* **49**: 465-479.

Bashan, Y., Salazar, B. and Puente, M.E. (2009). Responses of native legume desert trees used for reforestation in the Sonoran Desert to plant growth-promoting microorganisms in screen house. *Biol. Fert. soils.,* **45**: 655-662.

Bashan, Y., Salazar, B.G., Moreno, M., Lopez, B.R. and Linderman, R.G. (2012). Restoration of eroded soil in the Sonoran Desert with native leguminous trees using plant growth-promoting microorganisms and limited amounts of compost and water. *J. Environ. Manag.,* **102**: 26-36.

Bashour, I.I. and Sayegh, A.H. (2007). ***Methods of analysis for soils of arid and semi-arid regions***. FAO.

Batool, S. and Iqbal, A. (2018). Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of Triticum aestivum (Var. Galaxy 2013). *Saudi. J. Biol. Sci.,* **7**: 1400-1410.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B. and Sizmur, T. (2011). A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. pol.,* **159**: 3269-3282.

Benabderrahim, M.A., Elfalleh, W., Belayadi, H. and Haddad, M. (2018). Effect of date palm waste compost on forage alfalfa growth, yield, seed yield and minerals uptake. *Int. J. Recyc. Org. Waste Agric.,* **7**: 1-9.

Bender, S.F., Wagg, C. and van der Heijden, M.G. (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol. Evol.,* **31**: 440-452.

Benedek, S., Elfoughi, A., Abdorhim, H.A., Bayoumi, H.H. and Füleky, G. (2012). Effects of compost application on soil fertility of a Luvisol from Gödöllő (Hungary). *Arch. Agron. Soil Sci.,* **58**: S103-S106.

Bennett, E.M., Peterson, G.D. and Gordon, L.J. (2009). Understanding relationships among multiple ecosystem services. *Ecol. Lett.,* **12**: 1394-1404.

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biot.,* **84**: 11-18.

Berg, G., Rybakova, D., Grube, M. and Köberl, M. (2015). The plant microbiome explored: implications for experimental botany. *J. Exper. Botany.,* **67**: 995-1002.

Berry, L. (2003). Land degradation in Ethiopia: Its extent and impact. *Comm. GM. WB support*: 2-7.

Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World. J. Microbiol. Biot.,* **28**: 1327-1350.

Bjelić, D., Marinković, J., Tintor, B. and Mrkovački, N. (2018). Antifungal and plant growth promoting activities of indigenous rhizobacteria isolated from maize (Zea mays L.) rhizosphere. *Commun. Soil Sci. Plant Analy.,* **49**: 88-98.

Bohre, P. and Chaubey, O. (2014). Restoration of degraded lands through plantation forests. *Glob. J. Sci. Fron. Res.: Biol. Sci.,* **14**: 18-27.

Borriss, R. (2011). **Use of plant-associated Bacillus strains as biofertilizers and biocontrol agents in agriculture**. **In**: *Bacteria in agrobiology: Plant growth responses*, pp. 41-76, Springer.

Bowers, J.E. and Turner, R.M. (2001). Dieback and episodic mortality of Cercidium microphyllum (foothill paloverde), a dominant Sonoran Desert tree. *J. Torrey Bot. Soc.*: 128-140.

Braga, L.F., de Oliveira, F.A., do Couto, E.A.P., Santos, K.F.d.E.N., de Brito Ferreira, E.P. and Martin-Didonet, C.C.G. (2018). Polyphasic characterization of bacteria obtained from upland rice cultivated in Cerrado soil. *Braz. J. Microbiol.,* **49**: 20-28.

Brook, B.W., Ellis, E.C., Perring, M.P., Mackay, A.W. and Blomqvist, L. (2013). Does the terrestrial biosphere have planetary tipping points? *Tren. Ecol. Evol.,* **28**: 396-401.

Brook, B.W., Sodhi, N.S. and Bradshaw, C.J. (2008). Synergies among extinction drivers under global change. *Tren. Ecol. Evol.,* **23**: 453-460.

Brückner, R. and Titgemeyer, F. (2002). Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. *FEMS Microbiol. Let.,* **209**: 141-148.

Bruun, E.W., Ambus, P., Egsgaard, H. and Hauggaard-Nielsen, H. (2012). Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. *Soil Biol. Biochem.,* **46**: 73-79.

Buckles, D., Triomphe, B. and Sain, G. (1998). ***Cover crops in hillside agriculture: farmer innovation with Mucuna***. International Development Research Centre, Ottawa, Canada, pp. 277.

Bunch, R. and Kadar, A. (2004). La mucuna en los sistemas de agricultura de bajos insumos externos en Mesoamérica. *Revista Leisa de Agroecología, junio*: 16-18.

Byers, A.-K., Condron, L., Donavan, T., O'Callaghan, M., Patuawa, T., Waipara, N. and Black, A. (2020). Soil microbial diversity in adjacent forest systems–contrasting native, old growth kauri (Agathis australis) forest with exotic pine (Pinus radiata) plantation forest. *FEMS Microbiol. Ecol.,* **96**: fiaa047.

Cakmakçi, R., Dönmez, F., Aydın, A. and Şahin, F. (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem.,* **38**: 1482-1487.

Calugay, R.J., Miyashita, H., Okamura, Y. and Matsunaga, T. (2003). Siderophore production by the magnetic bacterium Magnetospirillum magneticum AMB-1. *FEMS Microbiol. Lett.,* **218**: 371-375.

Caravaca, F., Alguacil, M., Azcón, R., Parladé, J., Torres, P. and Roldán, A. (2005). Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate, and Aspergillus niger. *Microb. Ecol.,* **49**: 73-82.

Caravaca, F., Barea, J., Figueroa, D. and Roldán, A. (2002). Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with Olea europaea subsp. sylvestris through changes in soil biological and physical parameters. *Appl Soil Ecol,* **20**: 107-118.

Carmo, D.L.d., Lima, L.B.d. and Silva, C.A. (2016). Soil fertility and electrical conductivity affected by organic waste rates and nutrient inputs. *Revista Brasileira de Ciência do Solo,* **40**: 1-17.

Cayuela, M., Van Zwieten, L., Singh, B., Jeffery, S., Roig, A. and Sánchez-Monedero, M. (2014). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agric., Ecosyst. Environ.,* **191**: 5-16.

Cerdà, A., González-Pelayo, Ó., Giménez-Morera, A., Jordán, A., Pereira, P., Novara, A., Brevik, E.C., Prosdocimi, M., Mahmoodabadi, M. and Keesstra, S. (2016). Use of barley straw residues to avoid high erosion and runoff rates on persimmon plantations in Eastern Spain under low frequency–high magnitude simulated rainfall events. *Soil Res.,* **54**: 154-165.

Cernansky, R. (2015). Agriculture: State-of-the-art soil. *Nature News,* **517**: 258.

Chagnon, M., Kreutzweiser, D., Mitchell, E.A., Morrissey, C.A., Noome, D.A. and Van der Sluijs, J.P. (2015). Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ. Sci. Pollut. Res.,* **22**: 119-134.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A. and Joseph, S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Soil Research,* **45**: 629-634.

Chang, Y., Rossi, L., Zotarelli, L., Gao, B., Shahid, M.A. and Sarkhosh, A. (2021). Biochar improves soil physical characteristics and strengthens root architecture in Muscadine grape (Vitis rotundifolia L.). *Chemical and Biological Technologies in Agriculture,* **8**: 1-11.

Charest, M.-H., Beauchamp, C.J. and Antoun, H. (2005). Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and Pythium ultimum. *FEMS Microbiol. Ecol.,* **52**: 219-227.

Chasek, P., Safriel, U., Shikongo, S. and Fuhrman, V.F. (2015). Operationalizing zero net land degradation: The next stage in international efforts to combat desertification? *J. Arid Environ.,* **112**: 5-13.

Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P.S. and Nautiyal, C.S. (2012). Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. *Microb. Ecol.,* **64**: 450-460.

Cheng, C.-H. and Lehmann, J. (2009). Ageing of black carbon along a temperature gradient. *Chemosphere,* **75**: 1021-1027.

Cheng, C.-H., Lehmann, J. and Engelhard, M.H. (2008). Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. *Geochim. Cosmochim. Acta,* **72**: 1598-1610.

Chintala, R., Owen, R., Kumar, S., Schumacher, T. and Malo, D. (2014). Biochar impacts on denitrification under different soil water contents. *World Cong. Soil Sci.,* **6**: 23-31.

Chodak, M., Gołębiewski, M., Morawska-Płoskonka, J., Kuduk, K. and Niklińska, M. (2015). Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. *Ann. Microbiiol.,* **65**: 1627-1637.

Chojniak, J., Wasilkowski, D., Plaza, G., Mrozik, A. and Brigmon, R. (2015). Application of Biolog Microarrays Techniques for characterization of functional diversity of microbial community in phenolic-contaminated water. *Int. J. Environ. Res.,* **9**: 785-794.

Classen, A.T., Sundqvist, M.K., Henning, J.A., Newman, G.S., Moore, J.A., Cregger, M.A., Moorhead, L.C. and Patterson, C.M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere,* **6**: 1-21.

Clewell, A., Aronson, J. and Winterhalder, K. (2004). The SER international primer on ecological restoration.

Clewell, A.F. and Aronson, J. (2013). **Overview**. **In**: *Ecological Restoration,* pp. 3-13, Springer.

Cogger, C.G. (2005). Potential compost benefits for restoration of soils disturbed by urban development. *Compost Sci. Util.,* **13**: 243-251.

Coleman, D.C. and Whitman, W.B. (2005). Linking species richness, biodiversity and ecosystem function in soil systems. *Pedobiologia,* **49**: 479-497.

Cookson, W.R., Murphy, D.V. and Roper, M.M. (2008). Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. *Soil Biol. Biochem.,* **40**: 763-777.

Cowie, A., Penman, T., Gorissen, L., Winslow, M., Lehmann, J., Tyrrell, T., Twomlow, S., Wilkes, A., Lal, R. and Jones, J. (2011). Towards sustainable land management in the drylands: scientific connections in monitoring and assessing dryland degradation, climate change and biodiversity. *Land Degrad. Dev.,* **22**: 248-260.

Cruz, J.A. and Cadiente, M.K.M. (2016). Assessment of potential plant growth promoting compounds produced in vitro by endophytic bacteria associated with nipa palm (Nypa fruticans). *Philipp. J. Crop. Sci.,* **41**: 74-82.

Daniel Mengistu, Woldeamlak Bewket and Lal, R. (2016). Conservation effects on soil quality and climate change adaptability of Ethiopian watersheds. *Land Degradation & Development,* **27**: 1603-1621.

Das, N. (2000). Lathyrus sativus in rainfed multiple cropping systems in West Bengal, Indiaa review. *Lathyrus Lathyrism Newsletter,* **1**: 25-27.

Das, P., Behera, B.K., Meena, D.K., Azmi, S.A., Chatterjee, S., Meena, K. and Sharma, A.P. (2015). Salt stress tolerant genes in halophilic and halotolerant bacteria: Paradigm for salt stress adaptation and osmoprotection. *Int. J. Curr. Microbiol. App. Sci,* **4**: 642-658.

Dastager, S.G., Deepa, C. and Pandey, A. (2011). Potential plant growth-promoting activity of Serratia nematodiphila NII-0928 on black pepper (Piper nigrum L.). *World J. Microbiol. Biotechnol.,* **27**: 259-265.

Davis, J. and Whiting, D. (2013). Choosing a soil amendment. Retrieved 3 June 2013.

De-Bashan, L.E., Hernandez, J.-P., Bashan, Y. and Maier, R.M. (2010a). Bacillus pumilus ES4: candidate plant growth-promoting bacterium to enhance establishment of plants in mine tailings. *Environ. Exp. Bot.,* **69**: 343-352.

De-Bashan, L.E., Hernandez, J.-P., Nelson, K.N., Bashan, Y. and Maier, R.M. (2010b). Growth of quailbush in acidic, metalliferous desert mine tailings: effect of Azospirillum brasilense Sp6 on biomass production and rhizosphere community structure. *Microb. Ecol.,* **60**: 915-927.

de Freitas, A.D.S., Vieira, C.L., de Rosália, C.E., Santos, S. and Stamford, N.P. (2007). Caracterização de rizóbios isolados de Jacatupé cultivado em solo salino do estado de Pernambuco, Brasil. *Bragantia,* **66**: 497-504.

De Meyer, A., Poesen, J., Isabirye, M., Deckers, J. and Raes, D. (2011). Soil erosion rates in tropical villages: a case study from Lake Victoria Basin, Uganda. *Catena,* **84**: 89-98.

de Varennes, A., Cunha-Queda, C. and Qu, G. (2010). Amendment of an acid mine soil with compost and polyacrylate polymers enhances enzymatic activities but may change the distribution of plant species. *Water, Air, Soil Pollut.,* **208**: 91-100.

del Carmen Jaizme-Vega, M., Rodríguez-Romero, A.S. and Guerra, M.S.P. (2004). Potential use of rhizobacteria from the Bacillus genus to stimulate the plant growth of micropropagated bananas. *Fruits,* **59**: 83-90.

del Mar Alguacil, M., Díaz-Pereira, E., Caravaca, F., Fernández, D.A. and Roldán, A. (2009). Increased diversity of arbuscular mycorrhizal fungi in a long-term field experiment via application of organic amendments to a semiarid degraded soil. *Applied and Environmental Microbiology,* **75**: 4254-4263.

Deng, H., Ge, L., Xu, T., Zhang, M., Wang, X., Zhang, Y. and Peng, H. (2011). Analysis of the metabolic utilization of carbon sources and potential functional diversity of the bacterial community in lab-scale horizontal subsurface-flow constructed wetlands. *J. Environ. Qual.,* **40**: 1730-1736.

Ding, G.-C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., Carrillo, A., Andersen, G.L., Castellanos, T., Tebbe, C.C. and Smalla, K. (2013). Changes of soil bacterial diversity as a consequence of agricultural land use in a semi-arid ecosystem. *PLoS One,* **8**: e59497.

Dinnes, D.L., Karlen, D.L., Jaynes, D.B., Kaspar, T.C., Hatfield, J.L., Colvin, T.S. and Cambardella, C.A. (2002). Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils. *Agron. J.,* **94**: 153-171.

Diriba Muleta. (2007). ***Microbial inputs in coffee (Coffea arabica L.) production systems, Southwestern Ethiopia***. pp. 1-67.

Diriba Muleta, Fassil Assefa, Hjort, K., Roos, S. and Granhall, U. (2009). Characterization of Rhizobacteria isolated from Wild Coffea arabica L. *Eng. Life. Sci.,* **9**: 100-108.

Dodor, D.E., Amanor, Y.J., Attor, F.T., Adjadeh, T.A., Neina, D. and Miyittah, M. (2018). Co-application of biochar and cattle manure counteract positive priming of carbon mineralization in a sandy soil. *Environ. Syst Res.,* **7**: 5.

Dong, W.-Y., Zhang, X.-Y., Dai, X.-Q., Fu, X.-L., Yang, F.-T., Liu, X.-Y., Sun, X.-M., Wen, X.-F. and Schaeffer, S. (2014). Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Appl Soil Ecol*, **84**: 140-147.

Dubovyk, O. (2017). The role of Remote Sensing in land degradation assessments: opportunities and challenges. *Eur. J. Remote Sens.*, **50**: 601-613.

Duponnois, R. and Plenchette, C. (2003). A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian Acacia species. *Mycorrhiza,* **13**: 85-91.

Economist, W.B.A.R.O.O.o.t.C., Quality, W.B.A.R.O.O., Services, K. and Group, W.B.D.D. (2006). ***Africa development indicators***. World Bank Publications.

Edward, I. (2000). Applied Ecology and Environmental Management, Blackwell Publishing Company, Australia, England, pp. 1-6.

Egamberdieva, D. and Kucharova, Z. (2009). Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biol. Fertility Soils,* **45**: 563-571.

Eisenstein, M. (2013). Plant breeding: Discovery in a dry spell. *Nature,* **501**: S7-S9.

Elouear, Z., Bouhamed, F., Boujelben, N. and Bouzid, J. (2016). Application of sheep manure and potassium fertilizer to contaminated soil and its effect on zinc, cadmium and lead accumulation by alfalfa plants. *Sustain. Environ. Res.,* **26**: 131-135.

Emiru Birhane, Demel Teketay and Barklund, P. (2007). Enclosures to enhance woody species diversity in the dry lands of eastern Tigray, Ethiopia. *East Afr. J. Sci.,* **1**: 136-147.

Eni, D.I., Upla, J.I., Oko, C.O., Obiefuna, J.N. and Njar, G. (2010). Effects of land degradation on soil productivity in Calabar South Local Government Area, Nigeria. *Eur. J. Soc. Sci.,* **18**: 166-171.

Erdmann, T.K. (2005). **Agroforestry as a tool for restoring forest landscapes**. **In**: *Forest Restoration in Landscapes*, pp. 274-284, Springer.

Escalas, A., Hale, L., Voordeckers, J.W., Yang, Y., Firestone, M.K., Alvarez-Cohen, L. and Zhou, J. (2019). Microbial functional diversity: From concepts to applications. *Ecol. Evol.,* **9**: 12000-12016.

Esitken, A., Ercisli, S., Karlidag, H. and Sahin, F. (2005). Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. **In:** *Proceeding Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production*, pp. 90-97.

Eviner, V.T. and Hawkes, C.V. (2008). Embracing variability in the application of plant–soil interactions to the restoration of communities and ecosystems. *Restor. Ecol.,* **16**: 713-729.

Faisal, M. (2013). Inoculation of plant growth promoting bacteria Ochrobactrum intermedium, Brevibacterium sp. and Bacillus cereus induce plant growth parameters. *J. Appl. Biotech,* **1**: 45-53.

Fakruddin, M., Mannan, B., Shahnewaj, K., Mazumdar, R.M., Chowdhury, A. and Hossain, N. (2013). Identification and characterization of microorganisms: DNA-fingerprinting methods. *Songklanakarin Journal of Science & Technology,* **35 (4)**: 397-404.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for sustainable development,* **29**: 185-212.

Fasciglione, G., Casanovas, E.M., Quillehauquy, V., Yommi, A.K., Goñi, M.G., Roura, S.I. and Barassi, C.A. (2015). Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. *Scientia Horticulturae,* **195**: 154-162.

Fatemeh, A., Masoud, T., Pejman, A. and Aidin, H. (2014). Effect of plant growth promoting rhizobacteria (PGPRs) and stratification on germination traits of Crataegus pseudoheterophylla Pojark. seeds. *Scientia Horticulturae,* **172**: 61-67.

Felestrino, É.B., Santiago, I.F., Freitas, L.d.S., Rosa, L.H., Ribeiro, S.P. and Moreira, L.M. (2017). Plant Growth Promoting Bacteria Associated with Langsdorffia hypogaea-Rhizosphere-Host Biological Interface: A Neglected Model of Bacterial Prospection. *Front. Microbiol.,* **8**: 172.

Fellet, G., Marchiol, L., Delle Vedove, G. and Peressotti, A. (2011). Application of biochar on mine tailings: effects and perspectives for land reclamation. *Chemosphere,* **83**: 1262-1267.

Figueiredo, M., Martinez, C., Burity, H. and Chanway, C. (2008). Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (Phaseolus vulgaris L.). *World J. Microbiol. Biotechnol.,* **24**: 1187-1193.

Firew Elias, Delelegn Woyessa and Diriba Muleta. (2016). Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *Int. J. Microbiol.,* **2016**: 1-12.

Flores-Núñez, V.M., Amora-Lazcano, E., Rodríguez-Dorantes, A., Cruz-Maya, J.A. and Jan-Roblero, J. (2018). Comparison of plant growth-promoting rhizobacteria in a pine forest soil and an agricultural soil. *Soil Res.,* **56**: 346-355.

Forni, C., Duca, D. and Glick, B.R. (2017). Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. *Plant Soil,* **410**: 335-356.

Fraga, R., Rodriguez, H. and Gonzalez, T. (2001). Transfer of the gene encoding the NapA acid phosphatase of Morganella morganii to a Burkholderia cepacia strain. *Acta Biotechnol.,* **21**: 359-369.

Francis, I., Holsters, M. and Vereecke, D. (2010). The Gram-positive side of plant–microbe interactions. *Environ. Microbiol.,* **12**: 1-12.

Galinato, S.P., Yoder, J.K. and Granatstein, D. (2011). The economic value of biochar in crop production and carbon sequestration. *Energy Policy,* **39**: 6344-6350.

Garrity, D.P., Akinnifesi, F.K., Ajayi, O.C., Weldesemayat, S.G., Mowo, J.G., Kalinganire, A., Larwanou, M. and Bayala, J. (2010). Evergreen Agriculture: a robust approach to sustainable food security in Africa. *Food Secur.,* **2**: 197-214.

Gavili, E., Moosavi, A.A. and Moradi Choghamarani, F. (2018). Cattle manure biochar potential for ameliorating soil physical characteristics and spinach response under drought. *Arch. Agron. Soil Sci.,* **5**: 1-14.

Gebhardt, M.M. (2015). Soil amendment effects on degraded soils and consequences for plant growth and soil microbial communities.

Geetha, K., Venkatesham, E., Hindumathi, A. and Bhadraiah, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on Vigna Radita (L.) R. Wilczek. *Int. J. Curr. Microbiol. Appl. Sci.,* **3**: 799-899.

Genene Tsegaye. (2006). Farmers' Perceptions of Land Degradation and Determinants of Household Food Security Status at Middle Catchments of Bilate Watershed. *An MSc Thesis Presented to the School of Graduate Studies, Alemaya University*: 1-14.

Gennari, M., Abbate, C., Porta, V.L., Baglieri, A. and Cignetti, A. (2007). Microbial response to Na2SO4 additions in a volcanic soil. *Arid Land Res. Manag.,* **21**: 211-227.

Getachew Agegnehu, Srivastava, A. and Bird, M.I. (2017). The role of biochar and biochar-compost in improving soil quality and crop performance: a review. *Appl Soil Ecol,* **119**: 156-170.

Gholami, A., Shahsavani, S. and Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *Int. J. Biol. Life Sci.,* **5**: 35-40.

Giongo, A., Beneduzi, A., Ambrosini, A., Vargas, L.K., Stroschein, M.R., Eltz, F.L., Bodanese-Zanettini, M.H. and Passaglia, L.M.P. (2010). Isolation and characterization of two plant growth-promoting bacteria from the rhizoplane of a legume (Lupinus albescens) in sandy soil. *Revista Brasileira de Ciência do Solo,* **34**: 361-369.

Girvan, M.S., Bullimore, J., Ball, A.S., Pretty, J.N. and Osborn, A.M. (2004). Responses of active bacterial and fungal communities in soils under winter wheat to different fertilizer and pesticide regimens. *Appl. Environ. Microbiol.,* **70**: 2692-2701.

Giuliani, G., Chatenoux, B., Benvenuti, A., Lacroix, P., Santoro, M. and Mazzetti, P. (2020). Monitoring land degradation at national level using satellite Earth Observation time-series data to support SDG15–exploring the potential of data cube. *Big Earth Data*: 1-20.

Glenn, E., Waugh, W., Moore, D., McKeon, C. and Nelson, S. (2001). Revegetation of an abandoned uranium millsite on the Colorado Plateau, Arizona. *J. Environ. Qual.,* **30**: 1154-1162.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.,* **41**: 109-117.

Glick, B.R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Scientifica,* **2012**.

Glick, B.R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.,* **169**: 30-39.

Glick, B.R., Cheng, Z., Czarny, J. and Duan, J. (2007a). **Promotion of plant growth by ACC deaminase-producing soil bacteria**. **In**: *New perspectives and approaches in plant growth-promoting Rhizobacteria research*, pp. 329-339, Springer.

Glick, B.R., Cheng, Z., Czarny, J. and Duan, J. (2007b). Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.,* **119**: 329-339.

Gond, S.K., Bergen, M.S., Torres, M.S., White, J.F. and Kharwar, R.N. (2015). Effect of bacterial endophyte on expression of defense genes in Indian popcorn against Fusarium moniliforme. *Symbiosis,* **66**: 133-140.

Gonzalez, A.J., Larraburu, E.E. and Llorente, B.E. (2015). Azospirillum brasilense increased salt tolerance of jojoba during in vitro rooting. *Industrial Crops and Products,* **76**: 41-48.

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R.K., Gowda, C.L. and Krishnamurthy, L. (2015). Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech,* **5**: 355-377.

Gou, W., Tian, L., Ruan, Z., Zheng, P., Chen, F., Zhang, L., Cui, Z., Zheng, P., Li, Z. and Gao, M. (2015). Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (Zea mays) by three plant growth promoting rhizobacteria (PGPR) strains. *Pak J Bot,* **47**: 581-586.

Grönemeyer, J.L., Burbano, C.S., Hurek, T. and Reinhold-Hurek, B. (2012). Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. *Plant Soil,* **356**: 67-82.

Grover, M., Ali, S.Z., Sandhya, V., Rasul, A. and Venkateswarlu, B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology,* **27**: 1231-1240.

Grover, M., Madhubala, R., Ali, S.Z., Yadav, S. and Venkateswarlu, B. (2014). Influence of Bacillus spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *J. Basic Microbiol.,* **54**: 951-961.

Gu, Y., Zhang, X., Tu, S. and Lindström, K. (2009). Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. *European Journal of Soil Biology,* **45**: 239-246.

Güereña, D.T., Lehmann, J., Thies, J.E., Enders, A., Karanja, N. and Neufeldt, H. (2015). Partitioning the contributions of biochar properties to enhanced biological nitrogen fixation in common bean (Phaseolus vulgaris). *Biol. Fertil. Soils,* **51**: 479-491.

Guiñazú, L.B., Andrés, J.A., Rovera, M., Balzarini, M. and Rosas, S.B. (2013). Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards Rhizoctonia sp. and Macrophomina sp. in vitro. *Eur. J. Soil Biol.,* **54**: 69-77.

Haase, D.L. and Davis, A.S. (2017). Developing and supporting quality nursery facilities and staff are necessary to meet global forest and landscape restoration needs. *Reforesta*: 69-93.

Hamilton, C.E., Bever, J.D., Labbé, J., Yang, X. and Yin, H. (2016). Mitigating climate change through managing constructed-microbial communities in agriculture. *Agr. Ecosyst. Environ.,* **216**: 304-308.

Hardoim, P.R., Van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M. and Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.,* **79**: 293-320.

Harris, J. (2009). Soil microbial communities and restoration ecology: facilitators or followers? *Sci.*, **325**: 573-574.

Hashem, A., Abd_Allah, E., Alqarawi, A., Al-Huqail, A. and Shah, M. (2016). Induction of osmoregulation and modulation of salt stress in Acacia gerrardii Benth. by arbuscular mycorrhizal fungi and Bacillus subtilis (BERA 71). *BioMed. Res. Int.,* **2016**: 1-11.

Hayat, R., Khalid, R., Ehsan, M., Ahmed, I., Yokota, A. and Ali, S. (2013). Molecular characterization of soil bacteria for improving crop yield in Pakistan. *Pak J Bot,* **45**: 1045-1055.

Hazell, P. and Wood, S. (2008). Drivers of change in global agriculture. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.,* **363**: 495-515.

Higginbottom, T.P. and Symeonakis, E. (2014). Assessing land degradation and desertification using vegetation index data: Current frameworks and future directions. *Remote Sens.,* **6**: 9552-9575.

Hirt, H. (2009). ***Plant stress biology: From genomics to systems biology***. John Wiley & Sons.

Hobbs, R.J. and Cramer, V.A. (2008). Restoration ecology: interventionist approaches for restoring and maintaining ecosystem function in the face of rapid environmental change. *Annu. Rev. Environ. Resour.,* **33**: 39-61.

Hossain, M.K., Strezov, V., Chan, K.Y., Ziolkowski, A. and Nelson, P.F. (2011). Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. *J. Environ. Manage.,* **92**: 223-228.

Huang, J., Yu, H., Guan, X., Wang, G. and Guo, R. (2016). Accelerated dryland expansion under climate change. *Nat. Clim. Chang.,* **6**: 166.

Huang, L., Baumgartl, T. and Mulligan, D. (2012). Is rhizosphere remediation sufficient for sustainable revegetation of mine tailings? *Ann. Bot.,* **110**: 223-238.

Huber, B., Scholz, H.C., Kämpfer, P., Falsen, E., Langer, S. and Busse, H.-J. (2010). Ochrobactrum pituitosum sp. nov., isolated from an industrial environment. *Int. J. Syst. Evol. Microbiol.,* **60**: 321-326.

Hueso-González, P., Muñoz-Rojas, M. and Martínez-Murillo, J. (2017). The role of organic amendments in drylands restoration. *Curr. Opin. Environ. Sci. Health,* **5**: 1-6.

Hung, M.-H., Bhagwath, A.A., Shen, F.-T., Devasya, R.P. and Young, C.-C. (2005). Indigenous rhizobia associated with native shrubby legumes in Taiwan. *Pedobiologia,* **49**: 577-584.

Hungria, M., Franchini, J.C., Brandao-Junior, O., Kaschuk, G. and Souza, R.A. (2009). Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. *Appl Soil Ecol,* **42**: 288-296.

Hungria, M. and Vargas, M.A. (2000). Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res.,* **65**: 151-164.

Hunter, P.R. and Gaston, M.A. (1988). Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol.,* **26**: 2465-2466.

Hurni, H. (1985). Erosion-productivity-conservation systems in Ethiopia. 654-674.

Hurni, K., Gete Zeleke, Minale Kassie, Berhan Tegegne, Tibebu Kassawmar, ErmiasTeferi, Aderajew Moges, Deme Tadesse, Mohamed Ahmed and Yohannes Degu. (2015). Economics of land degradation (ELD) Ethiopia case study. *Soil degradation and sustainable land management in the rainfed agricultural areas of Ethiopia: an assessment of the economic implications. Report for the Economics of Land Degradation Initiative*: 94.

Iqbal, M., Khan, A.G. and Amjad, M. (2012). Soil Physical Health Indices, Soil Organic Carbon, Nitrate Contents and Wheat Growth as Influenced by Irrigation and Nitrogen Rates. *Int. J. Agric. Biol.,* **14**.

ISO, N. (1998). Soil quality, determination of total nitrogen content by dry combustion (“elemental analysis”). *International Organization for Standardization, Geneve, Switzerland*.

ISO. (1994). ***Soil Quality: Determination of Phosphorus: Spectrometric Determination of Phosphorus Soluble in Sodium Hydrogen Carbonate Solution***. ISO.

ISO. (1995). ***Soil quality: Determination of organic and total carbon after dry combustion (elementary analysis)***. ISO.

Itävaara, M., Salavirta, H., Marjamaa, K. and Ruskeeniemi, T. (2016). **Geomicrobiology and metagenomics of terrestrial deep subsurface microbiomes**. **In**: *Advances in applied microbiology*, pp. 1-77), Vol. 94, Elsevier.

Iverson, S.L. and Maier, R.M. (2009). Effects of compost on colonization of roots of plants grown in metalliferous mine tailings, as examined by fluorescence in situ hybridization. *Appl. Environ. Microbiol.,* **75**: 842-847.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A. and Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Front. Plant Sci.,* **8**: 1617.

Jalal, M.A. and van der Helm, D. (2017). **Isolation and spectroscopic identification of fungal siderophores**. **In**: *Handbook of Microbial Iron Chelates (1991)*, pp. 235-270, CRC press.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C. and Whitman, W.B. (2010). Development of soil microbial communities during tallgrass prairie restoration. *Soil Biol. Biochem.,* **42**: 302-312.

Jie, C., Jing-Zhang, C., Man-Zhi, T. and Zi-tong, G. (2002). Soil degradation: a global problem endangering sustainable development. *J. Geogr. Sci.,* **12**: 243-252.

Jin, F., Ding, Y., Ding, W., Reddy, M., Fernando, W. and Du, B. (2011). Genetic diversity and phylogeny of antagonistic bacteria against Phytophthora nicotianae isolated from tobacco rhizosphere. *Int. J. Mol. Sci.,* **12**: 3055-3071.

Johri, B. (2001). Technology development and demonstration of a new bacterial inoculant (GRP3) for improved legume production. *Uttar Pradesh Govternment, Project report,* **4**: 1-12.

Joseph, B., Ranjan Patra, R. and Lawrence, R. (2012). Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L.). *Int. J. Plant Prod.,* **1**: 141-152.

Joshi, R. (2018). Tripartite plant microbe and nutrients interaction in the below ground soil ecosystems. *J. Medicinal Plants,* **6**: 36-40.

Joshi, V.D., Palei, N.N. and Rachh, P.R. (2009). Physicochemical properties of four farm site soils in area surrounding Rajkot, Gujrat, India. *International Journal of ChemTech Research,* **1**: 709-713.

Kanninen, M. (2010). Plantation forests: global perspectives. *Ecosystem goods and services from plantation forests*: 1-15.

Kardol, P. and Wardle, D.A. (2010). How understanding aboveground–belowground linkages can assist restoration ecology. *Trends Ecol. Evol.,* **25**: 670-679.

Kaushal, M. (2019). Microbes in cahoots with plants: MIST to hit the jackpot of agricultural productivity during drought. *Int. J. Mol. Sci.,* **20**: 1769.

Kaushal, M. and Wani, S.P. (2016). Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Annals of microbiology,* **66**: 35-42.

Kavamura, V.N., Santos, S.N., da Silva, J.L., Parma, M.M., Ávila, L.A., Visconti, A., Zucchi, T.D., Taketani, R.G., Andreote, F.D. and de Melo, I.S. (2013). Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiol. Res.,* **168**: 183-191.

Kazaz, S., Erbas, S. and Baydar, H. (2010). Breaking seed dormancy in oil rose (Rosa damascena Mill.) by microbial inoculation. *Afr. J. Biotechnol.,* **9**: 6503-6508.

Keesstra, S., Nunes, J., Novara, A., Finger, D., Avelar, D., Kalantari, Z. and Cerdà, A. (2018). The superior effect of nature based solutions in land management for enhancing ecosystem services. *Sci. Total Environ.,* **610**: 997-1009.

Keya, S., Mureria, N. and Arshad, M. (1982). Population dynamics of soil microorganisms in relation to proximity of termite mounds in Kenya. *J. Arid Environ.,* **5**: 353-360.

Khan, M.A., Khan, S., Khan, A. and Alam, M. (2017). Soil contamination with cadmium, consequences and remediation using organic amendments. *Sci. Total Environ.,* **601**: 1591-1605.

Khan, N., Bano, A., Rahman, M.A., Guo, J., Kang, Z. and Babar, M.A. (2019). Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (Cicer arietinum L.) induced by PGPR and PGRs. *Sci. Rep.,* **9**: 2097.

Khianngam, S., Pootaeng-on, Y., Techakriengkrai, T. and Tanasupawat, S. (2014). Screening and identification of cellulase producing bacteria isolated from oil palm meal. *J. Appl. Pharm. Sci.,* **4**: 90.

Kim, Y.-C., Glick, B.R., Bashan, Y. and Ryu, C.-M. (2012). **Enhancement of plant drought tolerance by microbes**. **In**: *Plant responses to drought stress*, pp. 383-413, Springer.

Kindeya Gebrehiwot. (2004). Dryland agro-forestry strategy for Ethiopia. *ICRAF, Nairobi-Kenya*: 1-26.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H. and Trevors, J.T. (2004). Methods of studying soil microbial diversity. *J. Microbiol. Methods,* **58**: 169-188.

Kiros Abay, SarahTewolde-Berhan and KassaTeka. (2020). The effect of exclosures on restoration of soil properties in Ethiopian lowland conditions. *Appl. Sci.,* **2**: 1-12.

Kirui, O. and Mirzabaev, A. (2015). Costs of land degradation in Eastern Africa. **In:** *Proceeding Costs of land degradation in Eastern Africa* Millan, Italy, pp. 1-38.

Kirui, O. and Mirzabaev, A. (2016). Cost of land degradation and improvement in Eastern Africa. **In:** *Proceeding Cost of land degradation and improvement in Eastern Africa* Millan, Italy, pp. 1-38.

Kisiel, A. and Kępczyńska, E. (2016). Medicago truncatula Gaertn. as a model for understanding the mechanism of growth promotion by bacteria from rhizosphere and nodules of alfalfa. *Planta,* **243**: 1169-1189.

Kloepper, J.W., Ryu, C.-M. and Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology,* **94**: 1259-1266.

Köberl, M., Müller, H., Ramadan, E.M. and Berg, G. (2011). Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. *PLoS One,* **6**: e24452.

Küçük, Ç., Kivanç, M. and Kinaci, E. (2006). Characterization of Rhizobium sp. isolated from bean. *Tur. J. Biol.,* **30**: 127-132.

Kumar, A., Maurya, B. and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (Triticum aestivum L.). *Biocatal. Agric. Biotechnol.,* **3**: 121-128.

Kumar, A., Sharma, S., Mishra, S. and Dames, J. (2015). Arbuscular mycorrhizal inoculation improves growth and antioxidative response of Jatropha curcas (L.) under Na2SO4 salt stress. *Plant Biosystems- Int. J. Plant Biol.,* **149**: 260-269.

Kumar, D.P. and D AP, S. (2012). Evaluation of extracellular lytic enzymes from indigenous Bacillus isolates. *J. Microbiol. Biotechnol. Res,* **2**: 129-137.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.,* **33**: 1870-1874.

Kumar, V., Menon, S., Agarwal, H. and Gopalakrishnan, D. (2017). Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. *Resour.Efficient Technol.,* **3**: 434-439.

Kumari, B., Ram, M. and Mallaiah, K. (2010). Studies on nodulation, biochemical analysis and protein profiles of Rhizobium isolated from Indigofera species. *Malay. J. Microbiol.,* **6**: 133-139.

Lal, R. (2009). Soil degradation as a reason for inadequate human nutrition. *Food Security,* **1**: 45-57.

Lal, R. (2015a). Restoring soil quality to mitigate soil degradation. *Sust.,* **7**: 5875-5895.

Lal, R., Hall, G. and Miller, F. (1989). Soil degradation: I. Basic processes. *Land Degradation & Development,* **1**: 51-69.

Lambin, E.F. and Meyfroidt, P. (2011). Global land use change, economic globalization, and the looming land scarcity. *Proceedings of the National Academy of Sciences,* **108**: 3465-3472.

Larimer, A.L., Bever, J.D. and Clay, K. (2010). The interactive effects of plant microbial symbionts: a review and meta-analysis. *Symbiosis,* **51**: 139-148.

Larkin, M.A., Blackshields, G., Brown, N., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A. and Lopez, R. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics,* **23**: 2947-2948.

Larney, F.J. and Angers, D.A. (2012). The role of organic amendments in soil reclamation: A review. *Can. J. Soil Sci.,* **92**: 19-38.

Le, Q.B., Nkonya, E. and Mirzabaev, A. (2016). **Biomass productivity-based mapping of global land degradation hotspots**. **In**: *Economics of land degradation and improvement–A global assessment for sustainable development*, pp. 55-84, Springer.

Leal-Klevezas, D.S., Martínez-de-la-Vega, O., Ramírez-Barba, E.J., Osterman, B., Martínez-Soriano, J.P. and Simpson, J. (2005). Genotyping of Ochrobactrum spp. by AFLP analysis. *J. Bacteriol.,* **187**: 2537-2539.

Lee, S., Najiah, M., Wendy, W., Zahrol, A. and Nadirah, M. (2009). Multiple antibiotic resistance and heavy metal resistance profile of bacteria isolated from giant freshwater prawn (Macrobrachium rosenbergii) hatchery. *Agric Sci Chin,* **8**: 740-745.

Lehmann, J. and Joseph, S. (2015). ***Biochar for environmental management: science, technology and implementation***. Routledge, Melbourne.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. and Crowley, D. (2011). Biochar effects on soil biota–a review. *Soil biology and biochemistry,* **43**: 1812-1836.

León, J.D. and Osorio, N.W. (2014). Role of litter turnover in soil quality in tropical degraded lands of Colombia. *Sci. World J.,* **2014**: 1-11.

Lesk, C., Rowhani, P. and Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature,* **529**: 84-87.

Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytol,* **171**: 501-523.

Lewis, D.E., White, J.R., Wafula, D., Athar, R., Dickerson, T., Williams, H.N. and Chauhan, A. (2010). Soil functional diversity analysis of a bauxite-mined restoration chronosequence. *Microb. Ecol.,* **59**: 710-723.

Leyva, L.A. and Bashan, Y. (2008). Activity of two catabolic enzymes of the phosphogluconate pathway in mesquite roots inoculated with Azospirillum brasilense Cd. *Plant Physiol. Biochem.,* **46**: 898-904.

Li, H.-B., Singh, R.K., Singh, P., Song, Q.-Q., Xing, Y.-X., Yang, L.-T. and Li, Y.-R. (2017). Genetic diversity of nitrogen-fixing and plant growth promoting Pseudomonas species isolated from sugarcane rhizosphere. *Front. Microbiol.,* **8**: 1268.

Li, L., Xu, M., Eyakub Ali, M., Zhang, W., Duan, Y. and Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS One*, **13**: e0203812.

Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S. and Litvin, S. (2010). Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. *Int. J. Environ. Sci. Technol. (Tehran),* **7**: 307-312.

Lionard, M., Péquin, B., Lovejoy, C. and Vincent, W.F. (2012). Benthic cyanobacterial mats in the high arctic: multi-layer structure and fluorescence responses to osmotic stress. *Front. Microbiol.,* **3**: 140.

Liu, F., Ma, H., Peng, L., Du, Z., Ma, B. and Liu, X. (2019). Effect of the inoculation of plant growth-promoting rhizobacteria on the photosynthetic characteristics of Sambucus williamsii Hance container seedlings under drought stress. *AMB Express,* **9**: 1-9.

Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B. and Glaser, B. (2012). Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. *J. Plant Nutr. Soil Sci.,* **175**: 698-707.

Liu, X.-L., WANG, C., WU, F., XUE, D.-H. and CHEN, K. (2006). Studies on tobacco rhizosphere microbes. *Acta. Ecol. Sin.,* **26**: 552-557.

Lixia, Z., Weimin, Y., Zhigang, Y., Zhian, L. and Mingmao, D. (2004). Soil microbial characteristics in rehabilitation process of degraded ecosystems in Heshan. *J. Trop. Subtrop. Bot.,* **12**: 202-206.

Lone, P.A., Bhardwaj, A.K., Shah, K.W. and Bahar, F.A. (2016). Assessment of Soil Macronutrient Status of Some Threatened Medicinal Plants of Kashmir Himalaya, India. *Research Journal of Botany,* **11**: 18-24.

López-Ballesteros, A., Oyonarte, C., Kowalski, A.S., Serrano-Ortiz, P., Sánchez-Cañete, E.P., Moya, M.R. and Domingo, F. (2018). Can land degradation drive differences in the C exchange of two similar semiarid ecosystems? *Biogeosciences,* **15**: 263–278,.

Lori, M., Symnaczik, S., Mäder, P., De Deyn, G. and Gattinger, A. (2017). Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression. *PLoS One,* **12**: e0180442.

Louws, F.J., Fulbright, D.W., Stephens, C.T. and De Bruijn, F. (1994). Specific genomic fingerprints of phytopathogenic Xanthomonas and Pseudomonas pathovars and strains generated with repetitive sequences and PCR. *Appl. Environ. Microbiol.,* **60**: 2286-2295.

Low, P. (2013). Economic and Social impacts of desertification, land degradation and drought. White Paper I. **In:** *Proceeding Economic and Social impacts of desertification, land degradation and drought. White Paper I* Germany, pp. 1-42.

Lucy, M., Reed, E. and Glick, B.R. (2004). Applications of free living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek,* **86**: 1-25.

Lugtenberg, B. and Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual review of microbiology,* **63**: 541-556.

Lukac, M., Grenni, P. and Gamboni, M. (2017). ***Soil biological communities and ecosystem resilience***. Springer, Rome, Italy.

Luna, L., Pastorelli, R., Bastida, F., Hernández, T., García, C., Miralles, I. and Solé-Benet, A. (2016). The combination of quarry restoration strategies in semiarid climate induces different responses in biochemical and microbiological soil properties. *Appl Soil Ecol,* **107**: 33-47.

Lyons, K.G., Brigham, C., Traut, B. and Schwartz, M.W. (2005). Rare species and ecosystem functioning. *Conserv. Biol.,* **19**: 1019-1024.

Ma, Y., Vosátka, M. and Freitas, H. (2019). Beneficial microbes alleviate climatic stresses in plants. *Front. Plant Sci.,* **10**: 595.

Madsen, E.L. (2011). Microorganisms and their roles in fundamental biogeochemical cycles. *Curr. Opin. Biotechnol.,* **22**: 456-464.

Maestre, F.T., Sole, R. and Singh, B.K. (2017). Microbial biotechnology as a tool to restore degraded drylands. *Microb. Biotechnol.,* **10**: 1250-1253.

Mafia, R.G., Alfenas, A.C., Ferreira, E.M., Binoti, D.H.B., Mafia, G.M.V. and Mounteer, A.H. (2009). Root colonization and interaction among growth promoting rhizobacteria isolates and eucalypts species. *Rev Arvore,* **33**: 1-9.

Maitima, J.M., Mugatha, S.M., Reid, R.S., Gachimbi, L.N., Majule, A., Lyaruu, H., Pomery, D., Mathai, S. and Mugisha, S. (2009). The linkages between land use change, land degradation and biodiversity across East Africa. *Afr. J. Environ. Sci. Technol.,* **3**.

Major, J., Rondon, M., Molina, D., Riha, S.J. and Lehmann, J. (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil,* **333**: 117-128.

Maltas, A., Kebli, H., Oberholzer, H.R., Weisskopf, P. and Sinaj, S. (2018). The effects of organic and mineral fertilizers on carbon sequestration, soil properties, and crop yields from a long-term field experiment under a Swiss conventional farming system. *Land Degrad. Dev.,* **29**: 926-938.

Manasa, K., Subhash, R. and Trivenu, S. (2017). In vitro screening of temperature stress tolerance of Rhizobial and Pseudomonas fluorescence isolates. *J. Pharmaco. Phytochem,* **6**: 764-767.

Manoj, K., Mishra, S., Dixit, V., Kumar, M., Agarwal, L., Chauhan, P.S. and Nautiyal, C.S. (2016). Synergistic effect of Pseudomonas putida and Bacillus amyloliquefaciens ameliorates drought stress in chickpea (Cicer arietinum L.). *Plant signal. Behav.,* **11**: e1071004.

Mapelli, F., Marasco, R., Rolli, E., Barbato, M., Cherif, H., Guesmi, A., Ouzari, I., Daffonchio, D. and Borin, S. (2013). Potential for plant growth promotion of rhizobacteria associated with Salicornia growing in Tunisian hypersaline soils. *BioMed. Res. Int.,* **2013**.

Marble, A. and Fritschel, H. (2014). 2013 Global Food Policy Report, International Food Policy Research Institute. *2013 Global Food Policy Report, International Food Policy Research Institute.*

Margesin, R. and Schinner, F. (2005). ***Manual for soil analysis-monitoring and assessing soil bioremediation***. Springer Science & Business Media, SBerlin, Heidelberg.

Marques, A.P., Pires, C., Moreira, H., Rangel, A.O. and Castro, P.M. (2010). Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. *Soil Biol. Biochem.,* **42**: 1229-1235.

Martin, D.M. (2017). Ecological restoration should be redefined for the twenty-first century. *Restor. Ecol.,* **25**: 668-673.

Marulanda, A., Azcón, R., Chaumont, F., Ruiz-Lozano, J.M. and Aroca, R. (2010). Regulation of plasma membrane aquaporins by inoculation with a Bacillus megaterium strain in maize (Zea mays L.) plants under unstressed and salt-stressed conditions. *Planta,* **232**: 533-543.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D., Fatma, T. and Rattan, A. (2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian J. Med. Microbiol.,* **24**: 25.

Matos, A., Kerkhof, L. and Garland, J. (2005). Effects of microbial community diversity on the survival of Pseudomonas aeruginosa in the wheat rhizosphere. *Microb. Ecol.,* **49**: 257-264.

Maurice Bilung, L., Sin Chai, L., Tahar, A.S., Ted, C.K. and Apun, K. (2018). Prevalence, genetic heterogeneity, and antibiotic resistance profile of listeria spp. and Listeria monocytogenes at farm level: a highlight of ERIC-and BOX-PCR to reveal genetic diversity. *BioMed Res. Int.,* **2018**: 1-13.

Mayak, S., Tirosh, T. and Glick, B.R. (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.,* **42**: 565-572.

Mazur, A., Stasiak, G., Wielbo, J., Koper, P., Kubik-Komar, A. and Skorupska, A. (2013). Phenotype profiling of Rhizobium leguminosarum bv. trifolii clover nodule isolates reveal their both versatile and specialized metabolic capabilities. *Arch. Microbiol.,* **195**: 255-267.

Medina, A. and Azcón, R. (2010). Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions. *J. soil Sci. plant nut.,* **10**: 354-372.

Medina, A., Vassileva, M., Caravaca, F., Roldán, A. and Azcón, R. (2004). Improvement of soil characteristics and growth of Dorycnium pentaphyllum by amendment with agrowastes and inoculation with AM fungi and/or the yeast Yarowia lipolytica. *Chemosphere,* **56**: 449-456.

Mehta, P., Walia, A. and Shirkot, C. (2015). Functional diversity of phosphate solubilizing plant growth promoting rhizobacteria isolated from apple trees in the trans Himalayan region of Himachal Pradesh, India. *Biol. Agri. Hort.,* **31**: 265-288.

Melero, S., Madejón, E., Ruiz, J.C. and Herencia, J.F. (2007). Chemical and biochemical properties of a clay soil under dryland agriculture system as affected by organic fertilization. *European journal of agronomy*, **26**: 327-334.

Mendes, R., Garbeva, P. and Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.,* **37**: 634-663.

Mengistu Asmamaw. (2011). The role of area closures for soil and woody vegetation rehabilitation in Kewot District, North Shewa. Type of thesis MSc Thesis, Addis Ababa University, Ethiopia, pp. 1-76.

Mengual, C., Schoebitz, M., Azcón, R. and Roldán, A. (2014). Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. *J. Environ. Manag.,* **134**: 1-7.

Mengual, C., Schoebitz, M., Caravaca, F. and Roldán, A. (2016). Assessment of the potential role of Streptomyces strains in the revegetation of semiarid sites: the relative incidence of strain origin and plantation site on plant performance and soil quality indicators. *Biol. Fert. soils,* **52**: 53-64.

Menna, P., Pereira, A.A., Bangel, E.V. and Hungria, M. (2009). Rep-PCR of tropical rhizobia for strain fingerprinting, biodiversity appraisal and as a taxonomic and phylogenetic tool. *Symbiosis*, **48**: 120-130.

Mensah, A.K. and Frimpong, K.A. (2018). Biochar and/or Compost Applications Improve Soil Properties, Growth, and Yield of Maize Grown in Acidic Rainforest and Coastal Savannah Soils in Ghana. *International Journal of Agronomy*, **2018**.

Merkineh Mesene. (2017). Extent & Impact of Land Degradation and Rehabilitation Strategies: Ethiopian Highlands. *Global J. Human-Soc. Sci. Res.*, **12**: 1-17.

Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Oosterbaan, A., Tack, F.M. and Verheyen, K. (2007). Tree species effect on the redistribution of soil metals. *Environ. Pollut.*, **149**: 173-181.

Mesa, J., Mateos-Naranjo, E., Caviedes, M.A., Redondo-Gómez, S., Pajuelo, E. and Rodríguez-Llorente, I.D. (2015). Endophytic cultivable bacteria of the metal bioaccumulator Spartina maritima improve plant growth but not metal uptake in polluted marshes soils. *Front. Microbiol.*, **6**: 1450.

Meskerem Abi and Degefa Tolossa. (2015). Household food security status and its determinants in girar jarso woreda, north Shewa zone of oromia region, Ethiopia. *J. Sustain. Dev. Afr*, **17**: 118-137.

Messay Tefera. (2011). Land-use/land-cover dynamics in Nonno District, Central Ethiopia. *J. Sust. Develop. Afr.*, **13**: 123-141.

Messi, P., Guerrieri, E. and Bondi, M. (2005). Antibiotic resistance and antibacterial activity in heterotrophic bacteria of mineral water origin. *Sci. Total Environ.*, **346**: 213-219.

Mhete, M., Eze, P.N., Rahube, T.O. and Akinyemi, F.O. (2020). Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. *Scientific African*, **7**: e00246.

Mia, M.B., Shamsuddin, Z. and Mahmood, M. (2012). Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. *Afr. J. Biotechnol.*, **11**: 3758-3765.

Mia, S., Van Groenigen, J., Van de Voorde, T., Oram, N., Bezemer, T., Mommer, L. and Jeffery, S. (2014). Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability. *Agr. Ecosyst. Environ.*, **191**: 83-91.

Mikha, M.M., Benjamin, J.G., Stahlman, P.W. and Geier, P.W. (2014). Remediation/restoration of degraded soil: I. Impact on soil chemical properties. *Agron. J.*, **106**: 252-260.

Miller, J., Beasley, B., Drury, C., Larney, F. and Hao, X. (2015). Influence of long-term (9 yr) composted and stockpiled feedlot manure application on selected soil physical properties of a clay loam soil in southern Alberta. *Compost Sci. Util.*, **23**: 1-10.

Mishra, R.K., Mohammad, N. and Roychoudhury, N. (2015). Soil pollution: Causes, effects and control. *Tropical Forest Research Institute*, **3**: 20-30.

Mnasri, B., Aouani, M.E. and Mhamdi, R. (2007). Nodulation and growth of common bean (Phaseolus vulgaris) under water deficiency. *Soil Biol. Biochem.*, **39**: 1744-1750.

Mohammed Kasim, Zebene Assfaw, Abayneh Derero, Mateos Melkato and Yoseph Mamo. (2015). The role of area closure on the recovery of woody species composition on degraded lands and its socio-economic importance in central Rift valley area, Ethiopia. *Int. J. Dev. Res.,* **5**: 3348-3358.

Mohammed, M.A., Chernet, M.T. and Tuji, F.A. (2020). Phenotypic, stress tolerance, and plant growth promoting characteristics of rhizobial isolates of grass pea. *Int. Microbiol.,* **23**: 607-618.

Mohhammed, M., Kibebew, K. and Tekalign, M. (2016). Fertility mapping of some micronutrients in soils of Cheha District, Gurage Zone, Southern Ethiopia. *Afr. J. Soil. Sci.,* **4**: 313-320.

Montgomery, D.R. (2007). Soil erosion and agricultural sustainability. *Proc. Natl. Acad. Sci.,* **104**: 13268-13272.

Montoya-Tangarife, C., De La Barrera, F., Salazar, A. and Inostroza, L. (2017). Monitoring the effects of land cover change on the supply of ecosystem services in an urban region: A study of Santiago-Valparaíso, Chile. *PLoS One,* **12**: e0188117.

Muir, J., Pitman, W. and Foster, J. (2011). Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? *Grass Forage Sci.,* **66**: 301-315.

Mukherjee, A., Lal, R. and Zimmerman, A. (2014). Effects of biochar and other amendments on the physical properties and greenhouse gas emissions of an artificially degraded soil. *Sci. Total Environ.,* **487**: 26-36.

Mulualem Tigabu, M.L., Mesele Negash, and Demel Teketay. (2014). Rehabilitation of degraded forest and woodland ecosystems in Ethiopia for sustenance of livelihoods and ecosystem services. *IUFRO World Series,* **32**: 299-313.

Nadeem, S.M., Zahir, Z.A., Naveed, M. and Ashraf, M. (2010). Microbial ACC-deaminase: prospects and applications for inducing salt tolerance in plants. *Crit. Rev. Plant Sci.,* **29**: 360-393.

Najar, G.R., Ganie, M.A. and Tahir, A. (2015). Biochar for sustainable soil health: a review of prospects and concerns. *Pedosphere,* **25**: 639-653.

Nakhro, N. and Dkhar, M. (2010). Populations and biomass carbon in paddy field soil. *Agron. J.,* **9**: 102-110.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. *Eur. J. Soil Sci.,* **54**: 655-670.

Naseer, A. and Pandey, P. (2018). Assessment and monitoring of land degradation using geospatial technology in Bathinda district, Punjab, India. *Solid Earth,* **9**: 75.

Naveed, M., Mitter, B., Reichenauer, T.G., Wieczorek, K. and Sessitsch, A. (2014). Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. *Environ. Exp. Bot.,* **97**: 30-39.

Nazrul, M.I. and Shaheb, M.R. (2015). Adaptation of grass pea as sole and relay cropping systems with transplanted aman rice in Sylhet region of Bangladesh. *American J. Biol. Life Sci,* **3**: 254-259.

Nezarat, S. and Gholami, A. (2009). Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. *Pak. J. Biol. Sci.,* **12**: 26.

Ngom, A., Nakagawa, Y., Sawada, H., Tsukahara, J., Wakabayashi, S., Uchiumi, T., Nuntagij, A., Kotepong, S., Suzuki, A. and Higashi, S. (2004). A novel symbiotic nitrogen-fixing member of the Ochrobactrum clade isolated from root nodules of Acacia mangium. *J. Gen. Appl. Microbiol.,* **50**: 17-27.

Ngoma, L., Esau, B. and Babalola, O.O. (2013). Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *Afr. J. Biotechnol.,* **12**.

Nguyen, B.T., Trinh, N.N., Le, C.M.T., Nguyen, T.T., Tran, T.V., Thai, B.V. and Le, T.V. (2018). The interactive effects of biochar and cow manure on rice growth and selected properties of salt-affected soil. *Archives of Agronomy and Soil Science*: 1-15.

Nies, D.H. (1992). Resistance to cadmium, cobalt, zinc, and nickel in microbes. *Plasmid,* **27**: 17-28.

Nigussie Haregeweyn, Tsunekawa, A., Nyssen, J., Poesen, J., Tsubo, M., Meshesha Tsegaye, Schütt, B., Enyew Adgo and FirewT egegne. (2015). Soil erosion and conservation in Ethiopia: a review. *Prog. Phys. Geogr.,* **39**: 750-774.

Nkonya, E., von Braun, J., Mirzabaev, A., Le, Q.B., Kwon, H.Y. and Kirui, O. (2013). Economics of land degradation initiative: Methods and approach for global and national assessments.

Nobre, C.A., Sampaio, G., Borma, L.S., Castilla-Rubio, J.C., Silva, J.S. and Cardoso, M. (2016). Land-use and climate change risks in the Amazon and the need of a novel sustainable development paradigm. *Proceedings of the National Academy of Sciences,* **113**: 10759-10768.

Nunes, J., Araujo, A., Nunes, L., Lima, L., Carneiro, R., Salviano, A. and Tsai, S. (2012). Impact of land degradation on soil microbial biomass and activity in Northeast Brazil. *Pedosphere,* **22**: 88-95.

Nziguheba, G., Palm, C.A., Buresh, R.J. and Smithson, P.C. (1998). Soil phosphorus fractions and adsorption as affected by organic and inorganic sources. *Plant Soil,* **198**: 159-168.

Odoemelam, S. and Ajunwa, O. (2017). Heavy metal status and physicochemical properties of agricultural soil amended by short term applicaton of animal manure. *Curr. World Environ.,* **3**.

Ohsowski, B.M., Dunfield, K.E., Klironomos, J.N. and Hart, M.M. (2016). Improving plant biomass estimation in the field using partial least squares regression and ridge regression. *Botany,* **94**: 501-508.

Oren, A. and Garrity, G.M. (2014). Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. *Antonie Van Leeuwenhoek,* **106**: 43-56.

Palansooriya, K.N., Wong, J.T.F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S.X., Bolan, N., Wang, H. and Ok, Y.S. (2019). Response of microbial communities to biochar-amended soils: a critical review. *Biochar,* **1**: 3-22.

Pan, Y., Breidt, F. and Gorski, L. (2010). Synergistic effects of sodium chloride, glucose, and temperature on biofilm formation by Listeria monocytogenes serotype 1/2a and 4b strains. *Appl. Environ. Microbiol.*, **76**: 1433-1441.

Pandey, P.K., Samanta, R. and Yadav, R.N.S. (2015). Plant beneficial endophytic bacteria from the ethnomedicinal Mussaenda roxburghii (Akshap) of Eastern Himalayan Province, India. *Adv. Biol.*, **2015**.

Pant, G. and Agrawal, P.K. (2014). Isolation and characterization of indole acetic acid producing plant growth promoting rhizobacteria from rhizospheric soil of Withania somnifera. *J. Boil. Sci. Opin.*, **2**: 377-383.

Parham, J., Deng, S., Da, H., Sun, H. and Raun, W. (2003). Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. *Biology and Fertility of Soils*, **38**: 209-215.

Parrotta, J.A. (1992). The role of plantation forests in rehabilitating degraded tropical ecosystems. *Agric., Ecosyst. Environ.*, **41**: 115-133.

Pate, J.S. and Verboom, W.H. (2009). Contemporary biogenic formation of clay pavements by eucalypts: further support for the phytotarium concept. *Ann. Bot.*, **103**: 673-685.

Patten, C.L. and Glick, B.R. (2002). Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.*, **68**: 3795-3801.

Patwardhan, A., Ray, S. and Roy, A. (2014). Molecular markers in phylogenetic studies-a review. *J. Phylo. Evol. Biol.*, **2014**.

Paul, D. (2013). Osmotic stress adaptations in rhizobacteria. *J. Basic Microbiol.*, **53**: 101-110.

Paulitsch, F., Klepa, M.S., da Silva, A.R., do Carmo, M.R.B., Dall'Agnol, R.F., Delamuta, J.R.M., Hungria, M. and da Silva Batista, J.S. (2019). Phylogenetic diversity of rhizobia nodulating native Mimosa gymnas grown in a South Brazilian ecotone. *Mol. Biol. Rep.*, **46**: 529-540.

Paulucci, N.S., Gallarato, L.A., Reguera, Y.B., Vicario, J.C., Cesari, A.B., de Lema, M.B.G. and Dardanelli, M.S. (2015). Arachis hypogaea PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity. *Microbiol. Res.*, **173**: 1-9.

Pavinato, P.S. and Rosolem, C.A. (2008). Disponibilidade de nutrientes no solo: decomposição e liberação de compostos orgânicos de resíduos vegetais. *Revista Brasileira de Ciência do Solo*: 911-920.

Pender, J. and Berhanu Gebremedhin. (2007). Determinants of agricultural and land management practices and impacts on crop production and household income in the highlands of Tigray, Ethiopia. *J. Afr. Econ.*, **17**: 395-450.

Pender, J. and Gebremedhin, B. (2006). Land management, crop production, and household income in the highlands of Tigray, Northern Ethiopia: An econometric analysis. *Strategies for sustainable land management in the East African highlands*: 107-139.

Pender, J.L., Hagos, F. and Gebreselassie, N. (2012). Land degradation in the highlands of Tigray and strategies for sustainable land management.

Pérez-Fernández, M.A., Calvo-Magro, E. and Valentine, A. (2016). Benefits of the symbiotic association of shrubby legumes for the rehabilitation of degraded soils under Mediterranean climatic conditions. *Land degrad. Dev.,* **27**: 395-405.

Pieterse, C.M., Poelman, E., Van Wees, S. and Dicke, M. (2013). Induced plant responses to microbes and insects. *Front. Plant Sci.,* **4**: 475.

Pietikäinen, J., Kiikkilä, O. and Fritze, H. (2000). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos,* **89**: 231-242.

Piggot, P.J. and Hilbert, D.W. (2004). Sporulation of Bacillus subtilis. *Curr. Opin. Microbiol.,* **7**: 579-586.

Pistorius, T., Carodenuto, S. and Wathum, G. (2017). Implementing forest landscape restoration in Ethiopia. *Forests,* **8 (3)**: 1-19.

Premono, M.E., Moawad, A. and Vlek, P. (1996). Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere.

Prost, K., Borchard, N., Siemens, J., Kautz, T., Séquaris, J.-M., Möller, A. and Amelung, W. (2013). Biochar affected by composting with farmyard manure. *J. Environ. Qual.,* **42**: 164-172.

Quilliam, R.S., Glanville, H.C., Wade, S.C. and Jones, D.L. (2013). Life in the 'charosphere'–Does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biology and Biochemistry,* **65**: 287-293.

Ramachandran, A. and Radhapriya, P. (2016). Restoration of degraded soil in the Nanmangalam Reserve Forest with native tree species: effect of indigenous plant growth-promoting bacteria. *Sci. World J.,* **2016**: 1-10.

Ranjan, V., Sen, P., Kumar, D. and Sarsawat, A. (2015). A review on dump slope stabilization by revegetation with reference to indigenous plant. *Ecological Processes,* **4 (14)**: 1-11.

Rashid, M.I., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M. and Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiological research,* **183**: 26-41.

Rathnayake, I., Megharaj, M., Bolan, N. and Naidu, R. (2009). Tolerance of heavy metals by gram positive soil bacteria. Type of thesis World Academy of Science Engineering and Technology, pp. 1-5.

Reid, R.S., Serneels, S., Nyabenge, M. and Hanson, J. (2005). The changing face of pastoral systems in grass-dominated ecosystems of eastern Africa. *Grasslands of the World*: 19-76.

Reilly, T.J., Baron, G.S., Nano, F.E. and Kuhlenschmidt, M.S. (1996). Characterization and sequencing of a respiratory burst-inhibiting acid phosphatase from Francisella tularensis. *J. Biol. Chem.,* **271**: 10973-10983.

Reynolds, C.J., Piantadosi, J. and Boland, J. (2015). Rescuing food from the organics waste stream to feed the food insecure: an economic and environmental assessment of Australian food rescue operations using environmentally extended waste input-output analysis. *Sust.,* **7**: 4707-4726.

Ribeiro, C.M. and Cardoso, E.J.B.N. (2012). Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (Araucaria angustifolia). *Microbiol. Res.,* **167**: 69-78.

Rodríguez, H. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.,* **17**: 319-339.

Rodríguez, H., Fraga, R., Gonzalez, T. and Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil,* **287**: 15-21.

Rodriguez, H., Gonzalez, T., Goire, I. and Bashan, Y. (2004). Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium Azospirillum spp. *Naturwissenschaften,* **91**: 552-555.

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., Gandolfi, C., Casati, E., Previtali, F. and Gerbino, R. (2015). Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ. Microbiol.,* **17**: 316-331.

Romdhane, S.B., Trabelsi, M., Aouani, M.E., De Lajudie, P. and Mhamdi, R. (2009). The diversity of rhizobia nodulating chickpea (Cicer arietinum) under water deficiency as a source of more efficient inoculants. *Soil Biol. Biochem.,* **41**: 2568-2572.

Rossolini, G., Schippa, S., Riccio, M., Berlutti, F., Macaskie, L. and Thaller, M. (1998). Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology. *Cell. Mol. Life Sci. CMLS,* **54**: 833-850.

Rout, M.E. and Southworth, D. (2013). The root microbiome influences scales from molecules to ecosystems: The unseen majority1. *Am. J. Bot.,* **100**: 1689-1691.

Ruchi, K.R., Kumar, A., Patil, S., Thapa, S. and Kaur, M. (2012). Evaluation of plant growth promoting attributes and lytic enzyme production by fluorescent Pseudomonas diversity associated with apple and pear. *Int. J. Sci. Res. Pub.,* **2**: 2250-3153.

Rutigliano, F.A., Romano, M., Marzaioli, R., Baglivo, I., Baronti, S., Miglietta, F. and Castaldi, S. (2014). Effect of biochar addition on soil microbial community in a wheat crop. *Eur. J. Soil Biol.,* **60**: 9-15.

Sabet, K.K., Saber, M.M., El-Naggar, M.A.-A., El-Mougy, N.S., El-Deeb, H.M. and El-Shahawy, I.E.-S. (2013). Using commercial compost as control measures against cucumber root-rot disease. *J. Mycol.,* **2013**.

Sadasivam, S. and Manickam, A. (1992). ***Biochemical methods for agricultural sciences***. Wiley eastern limited.

Saharan, B. and Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sci. Med. Res.,* **21**: 30.

Sahay, H., Mahfooz, S., Singh, A.K., Singh, S., Kaushik, R., Saxena, A.K. and Arora, D.K. (2012). Exploration and characterization of agriculturally and industrially important haloalkaliphilic bacteria from environmental samples of hypersaline Sambhar lake, India. *World J. Microbiol. Biotechnol.,* **28**: 3207-3217.

Sahlemedhin Sertsu and Taye Bekele. (2000). Procedures for soil and plant analysis. *Ethiopian Agricultural Reaserch organization, Addis Ababa, Ethiopia*.

Saldaña, M.I., Gómez-Álvarez, R., Rivera-Cruz, M.d.C., Álvarez-Solís, J.D., Pat-Fernández, J.M. and Ortiz-García, C.F. (2014). The influence of organic fertilizers on the chemical properties of soil and the production of Alpinia purpurata. *Ciencia e investigación agraria,* **41**: 215-224.

Saleem, A.R., Brunetti, C., Khalid, A., Della Rocca, G., Raio, A., Emiliani, G., De Carlo, A., Mahmood, T. and Centritto, M. (2018). Drought response of Mucuna pruriens (L.) DC. inoculated with ACC deaminase and IAA producing rhizobacteria. *PLoS One,* **13**: e0191218.

Sánchez, P.A., Woomer, P.L. and Palm, C.A. (1994). Agroforestry approaches for rehabilitating degraded lands after tropical deforestation. **In:** *Proceeding Agroforestry approaches for rehabilitating degraded lands after tropical deforestation*, pp. 108-119.

Sandhya, V., Ali, S.Z., Grover, M., Reddy, G. and Venkateswarlu, B. (2010). Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regulation,* **62**: 21-30.

Sandhya, V., Grover, M., Reddy, G. and Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing Pseudomonas putida strain GAP-P45. *Biol. Fertility Soils,* **46**: 17-26.

Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P. and Samiyappan, R. (2011). Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta physiologiae plantarum,* **33**: 203-209.

Sarma, R.K. and Saikia, R. (2014). Alleviation of drought stress in mung bean by strain Pseudomonas aeruginosa GGRJ21. *Plant Soil,* **377**: 111-126.

Schillaci, M., Gupta, S., Walker, R. and Roessner, U. (2019). **The Role of Plant Growth-Promoting Bacteria in the Growth of Cereals under Abiotic Stresses**. **In**: *Root Biology-Growth, Physiology, and Functions*, IntechOpen.

Schröder, P., Beckers, B., Daniels, S., Gnädinger, F., Maestri, E., Marmiroli, N., Mench, M., Millan, R., Obermeier, M. and Oustriere, N. (2017). Intensify production, transform biomass to energy and novel goods and protect soils in Europe—a vision how to mobilize marginal lands. *Science of The Total Environment*.

Schroth, M. and Kloepper, J. (1978). Plant growth promoting rhizobacteria on radish. **In:** *Proceeding Plant growth promoting rhizobacteria on radish*, pp. 876-882.

Schwartz, M.W. and van Mantgem, P.J. (1997). **The value of small preserves in chronically fragmented landscapes**. **In**: *Conservation in highly fragmented landscapes,* pp. 379-394, Springer.

Scotti, R., Bonanomi, G., Scelza, R., Zoina, A. and Rao, M. (2015). Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *J. Soil Sci. Plant Nut.,* **15**: 333-352.

Seifu Tilahun, Yihenew G Selassie, Enyew Adgo, Kamaleddin Bashar, Ahmed Abdalla, Seleshi Awulachew, Easton, Z.M. and Steenhuis, T.S. (2013). **The Nile Basin sediment loss and**

degradation, with emphasis on the Blue Nile. **In**: *The Nile River Basin*, pp. 134-154, Routledge.

Shaik, I., Janakiram, P., Sujatha, L. and Ch, S. (2016). Isolation and identification of IAA producing endosymbiotic bacteria from Gracilaria corticata (J. Agardh). *International Journal of Bioassays,* **5**: 1-6.

Shanmugam, S.G., Magbanua, Z.V., Williams, M.A., Jangid, K., Whitman, W.B., Peterson, D.G. and Kingery, W.L. (2017). Bacterial diversity patterns differ in soils developing in sub-tropical and cool-temperate ecosystems. *Microb. Ecol.*, **73**: 556-569.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus,* **2**: 587.

Shi, S., Nan, L. and Smith, K.F. (2017). The current status, problems, and prospects of alfalfa (Medicago sativa L.) breeding in China. *agronomy,* **7**: 1.

Shilev, S. (2013). **Soil rhizobacteria regulating the uptake of nutrients and undesirable elements by plants**. **In**: *Plant microbe symbiosis: Fundamentals and advances,* pp. 147-167, Springer.

Shiomi, H.F. (2007). Bioprospecção de bactérias endofíticas como agentes de biocontrole da mancha de Exserohilum turcicum e como promotoras do crescimento de plantas de milho (Zea mays L.).

Shivlata, L. and Satyanarayana, T. (2017). **Actinobacteria in agricultural and environmental sustainability**. **In**: *Agro-Environmental Sustainability*, pp. 173-218, Springer.

Silva Filho, G.N. and Vidor, C. (2000). Solubilização de fostatos por microrganismos na presença de fontes de carbono. *Revista Brasileira de Ciência do Solo,* **24**: 311-319.

Silva, I.C.B.d., Basílio, J.J.N., Fernandes, L.A., Colen, F., Sampaio, R.A. and Frazão, L.A. (2017). Biochar from different residues on soil properties and common bean production. *Scientia Agricola,* **74**: 378-382.

Singh, J.S. (2015). Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. *Agriculture, ecosystems & environment,* **203**: 80-82.

Singh, J.S. and Gupta, V.K. (2018). Soil microbial biomass: a key soil driver in management of ecosystem functioning. *Sci. Total Environ.*, **634**: 497-500.

Singh, R.P., Bijo, A., Baghel, R.S., Reddy, C. and Jha, B. (2011). Role of bacterial isolates in enhancing the bud induction in the industrially important red alga Gracilaria dura. *FEMS Microbiol. Ecol.,* **76**: 381-392.

Sivasakthi, S., Usharani, G. and Saranraj, P. (2014). Biocontrol potentiality of plant growth promoting bacteria (PGPR)-Pseudomonas fluorescens and Bacillus subtilis: a review. *Afr. J. Agr. Res.,* **9**: 1265-1277.

Sneath, P.H. and Sokal, R.R. (1973). ***Numerical taxonomy. The principles and practice of numerical classification***.

Sohi, S.P., Krull, E., Lopez-Capel, E. and Bol, R. (2010). **A review of biochar and its use and function in soil**. **In**: *Advances in agronomy*, pp. 47-82), Vol. 105, Elsevier.

Solanki, M.K., Wang, Z., Wang, F.-Y., Li, C.-N., Lan, T.-J., Singh, R.K., Singh, P., Yang, L.-T. and Li, Y.-R. (2017). Intercropping in sugarcane cultivation influenced the soil properties and enhanced the diversity of vital diazotrophic bacteria. *Sugar Tech,* **19**: 136-147.

Solomon Abate. (1994). ***Land use dynamics, soil degradation and potential for sustainable use in Metu area, Illubabor region, Ethiopia***. University of Berne, Switzerland.

Solomon Legesse and Fassil Assefa (2014). Symbiotic and phenotypic characteristics of rhizobia nodulating faba bean (Vicia Faba) from Tahtay Koraro, northwestern zone of Tigray Regional State, Ethiopia. *Int. J. Emerg. Eng. Res. Technol,* **2**: 15-23.

Someya, N., Tsuchiya, K., Yoshida, T., Noguchi, M.T., Akutsu, K. and Sawada, H. (2007). Co-inoculation of an antibiotic-producing bacterium and a lytic enzyme-producing bacterium for the biocontrol of tomato wilt caused by Fusarium oxysporum f. sp. lycopersici. *Biocontrol Sci.,* **12**: 1-6.

Sonneveld, B. and Keyzer, M. (2003). Land under pressure: soil conservation concerns and opportunities for Ethiopia. *Land Degrad. Dev.,* **14**: 5-23.

Soumare, M., Tack, F. and Verloo, M. (2003). Effects of a municipal solid waste compost and mineral fertilization on plant growth in two tropical agricultural soils of Mali. *Bioresour. Technol.,* **86**: 15-20.

Souza, R.d., Ambrosini, A. and Passaglia, L.M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.,* **38**: 401-419.

Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.W., Ippolito, J.A., Collins, H.P., Boateng, A.A., Lima, I.M., Lamb, M.C. and McAloon, A.J. (2012). Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *J. Environ. Qual.,* **41**: 973-989.

Sridevi, M. and Mallaiah, K. (2009). Phosphate solubilization by Rhizobium strains. *Indian J. Microbiol.,* **49**: 98-102.

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E.L. and Lynch, S.V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS One,* **10**.

Stackebrandt, E. and Geobel, B.M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Evol. Microbiol.,* **44**: 846-849.

Steiner, C., Das, K., Melear, N. and Lakly, D. (2010). Reducing nitrogen loss during poultry litter composting using biochar. *J. Environ. Qual.,* **39**: 1236-1242.

Steiner, C., Garcia, M. and Zech, W. (2009). **Effects of charcoal as slow release nutrient carrier on NPK dynamics and soil microbial population: pot experiments with ferralsol substrate**. **In**: *Amazonian dark earths: Wim Sombroek's vision,* pp. 325-338, Springer.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E. and Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil,* **291**: 275-290.

Stuckey, H.T. and Hudak, P.F. (2001). Effects of compost on Loblolly Pine tree growth in Northeast Texas. *Compost Sci. Util.,* **9**: 65-72.

Subba Rao, N. (1982). Phosphate solubilization by soil microorganisms. *Advances in agricultural microbiology/edited by NS Subba Rao*.

Sunil, S.K., Pancholy, A., Jindal, S. and Pathak, R. (2011). Effect of plant growth promoting rhizobia on seed germination and seedling traits in Acacia senegal. *Ann. Forest Res.,* **54**: 161-169.

Suprapta, D.N. (2012). Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. *J ISSAAS,* **18**: 1-8.

Swaine, E., Swaine, M. and Killham, K. (2007). Effects of drought on isolates of Bradyrhizobium elkanii cultured from Albizia adianthifolia seedlings of different provenances. *Agroforestry systems,* **69**: 135-145.

Swift, M., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K., Kjoller, A., Loiseau, P. and Smith, P. (1998). Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. *Global Change Biol.,* **4**: 729-743.

Tagel Gebrehiwot and Veen, A. (2014). The effect of enclosures in rehabilitating degraded vegetation: a case of Enderta district, Northern Ethiopia. *Forest Res.: Open Access,* **3**: 1-15.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases. *Mol. Biol. Evol.,* **9**: 678-687.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.,* **30**: 2725-2729.

Tan, K. (1996). Measurement of field capacity water. *Soil sampling, preparation, and analysis. Marcel Dekker, Inc., New York*: 67-68.

Tansey, K., Grégoire, J.M., Defourny, P., Leigh, R., Pekel, J.F., Van Bogaert, E. and Bartholomé, E. (2008). A new, global, multi-annual (2000–2007) burnt area product at 1 km resolution. *Geophys. Res. Lett.,* **35**.

Tariq, M., Yasmin, S. and Hafeez, F.Y. (2010). Biological control of potato black scurf by rhizosphere associated bacteria. *Braz. J. Microbiol.,* **41**: 439-451.

Taschen, E., Sauve, M., Vincent, B., Parladé, J., van Tuinen, D., Aumeeruddy-Thomas, Y., Assenat, B., Selosse, M.-A. and Richard, F. (2020). Insight into the truffle brûlé: tripartite interactions between the black truffle (Tuber melanosporum), holm oak (Quercus ilex) and arbuscular mycorrhizal plants. *Plant Soil,* **446**: 577-594.

Tejada, M., Garcia, C., Gonzalez, J. and Hernandez, M. (2006). Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. *Soil Biol. Biochem.,* **38**: 1413-1421.

Tejada, M., Gómez, I., Hernández, T. and García, C. (2010). Utilization of vermicomposts in soil restoration: effects on soil biological properties. *Soil Sci. Soc. Am. J.,* **74**: 525-532.

Tesfa, A. and Mekuriaw, S. (2014). The effect of land degradation on farm size dynamics and crop-livestock farming system in ethiopia: A Review. *Open Journal of Soil Science,* **4**: 1.

Tewolde BG Egziabher. (1989). The environmental variables which led to the ecological crisis in Ethiopia. *Coenoses*: 61-67.

Teyssier, C., Marchandin, H., Jean-Pierre, H., Diego, I., Darbas, H., Jeannot, J.-L., Gouby, A. and Jumas-Bilak, E. (2005). Molecular and phenotypic features for identification of the opportunistic pathogens Ochrobactrum spp. *J. Med. Microbiol.*, **54**: 945-953.

Theobald, D.M. (2010). Estimating natural landscape changes from 1992 to 2030 in the conterminous US. *Landscape Ecol.,* **25**: 999-1011.

Thomas, S.C. and Gale, N. (2015). Biochar and forest restoration: a review and meta-analysis of tree growth responses. *New Forests,* **46**: 931-946.

Tiessen, H., Cuevas, E. and Chacon, P. (1994). The role of soil organic matter in sustaining soil fertility. *Nature*, **371**: 783-785.

Timmusk, S. and Wagner, E.G.H. (1999). The plant-growth-promoting rhizobacterium Paenibacillus polymyxa induces changes in Arabidopsis thaliana gene expression: a possible connection between biotic and abiotic stress responses. *Mol. Plant-Microbe Interact.,* **12**: 951-959.

Tognetti, R., Cocozza, C. and Marchetti, M. (2013). Shaping the multifunctional tree: the use of Salicaceae in environmental restoration. *iForest-Biogeosciences and Forestry,* **6**: 37.

Tripathi, V., Edrisi, S.A., Chen, B., Gupta, V.K., Vilu, R., Gathergood, N. and Abhilash, P. (2017). Biotechnological Advances for Restoring Degraded Land for Sustainable Development. *Trends in biotechnology,* **35**: 847-859.

Trivedi, P., Singh, K., Pankaj, U., Verma, S.K., Verma, R.K. and Patra, D. (2017). Effect of organic amendments and microbial application on sodic soil properties and growth of an aromatic crop. *Ecol. Eng.,* **102**: 127-136.

Trujillo, M.E., Willems, A., Abril, A., Planchuelo, A.-M., Rivas, R., Ludena, D., Mateos, P.F., Martínez-Molina, E. and Velázquez, E. (2005). Nodulation of Lupinus albus by Strains of Ochrobactrum lupini sp. nov. *Appl. Environ. Microbiol.,* **71**: 1318-1327.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. (2000). Biodegradation of lignin in a compost environment: a review. *Bioresour. Technol.,* **72**: 169-183.

Tzamali, E., Poirazi, P., Tollis, I.G. and Reczko, M. (2011). A computational exploration of bacterial metabolic diversity identifying metabolic interactions and growth-efficient strain communities. *BMC Syst. Biol.,* **5**: 167.

Upadhyay, S.K., Singh, D.P. and Saikia, R. (2009). Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. *Curr. Microbiol.,* **59**: 489-496.

Uzoma, K., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A. and Nishihara, E. (2011). Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil use and management,* **27**: 205-212.

Van Bruggen, A.H. and Semenov, A.M. (2000). In search of biological indicators for soil health and disease suppression. *Appl Soil Ecol,* **15**: 13-24.

van de Voorde, T.F., van der Putten, W.H. and Bezemer, T.M. (2012). Soil inoculation method determines the strength of plant–soil interactions. *Soil Biol. Biochem.,* **55**: 1-6.

Van Der Heijden, M.G., Bardgett, R.D. and Van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters,* **11**: 296-310.

Van Loon, L. (2007). Plant responses to plant growth-promoting rhizobacteria. *Eur. J. Plant Pathol.,* **119**: 243-254.

Vargas, R., Kenney, A.M. and Bilinski, T. (2019). Variable Influences of Water Availability and Rhizobacteria on the Growth of Schizachyrium scoparium (Little Bluestem) at Different Ages. *Frontiers in microbiology,* **10**.

Velasco, J., Romero, C., López-Goñi, I., Leiva, J., Díaz, R. and Moriyón, I. (1998). Evaluation of the relatedness of Brucella spp. and Ochrobactrum anthropi and description of Ochrobactrum intermedium sp. nov., a new species with a closer relationship to Brucella spp. *Int. J. Syst. Evol. Microbiol.,* **48**: 759-768.

Velu, R.K. (2013). ***Microbiological research in agroecosystem management***. Springer.

Verbon, E.H. and Liberman, L.M. (2016). Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in plant science,* **21**: 218-229.

Verchot, L.V., Van Noordwijk, M., Kandji, S., Tomich, T., Ong, C., Albrecht, A., Mackensen, J., Bantilan, C., Anupama, K. and Palm, C. (2007). Climate change: linking adaptation and mitigation through agroforestry. *Mitig. Adapt. Atrat. GL.,* **12**: 901-918.

Veum, K., Kremer, R., Sudduth, K., Kitchen, N., Lerch, R., Baffaut, C., Stott, D., Karlen, D. and Sadler, E. (2015). Conservation effects on soil quality indicators in the Missouri Salt River Basin. *J Soil Water Conserv,* **70**: 232-246.

Vimal, S.R., Singh, J.S., Arora, N.K. and Singh, S. (2017). Soil-plant-microbe interactions in stressed agriculture management: a review. *Pedosphere,* **27**: 177-192.

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M. and SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol. Res.,* **184**: 13-24.

Walker, D.J. and Bernal, M.P. (2008). The effects of olive mill waste compost and poultry manure on the availability and plant uptake of nutrients in a highly saline soil. *Bioresour. Technol.,* **99**: 396-403.

Walker, V., Couillerot, O., Von Felten, A., Bellvert, F., Jansa, J., Maurhofer, M., Bally, R., Moënne-Loccoz, Y. and Comte, G. (2012). Variation of secondary metabolite levels in maize seedling roots induced by inoculation with Azospirillum, Pseudomonas and Glomus consortium under field conditions. *Plant and soil,* **356**: 151-163.

Wallace, K.J. (2007). Classification of ecosystem services: problems and solutions. *Biol. Conserv.,* **139**: 235-246.

Wang, C., Wang, H., Li, Y., Li, Q., Yan, W., Zhang, Y., Wu, Z. and Zhou, Q. (2021). Plant growth-promoting rhizobacteria isolation from rhizosphere of submerged macrophytes and their growth-promoting effect on Vallisneria natans under high sediment organic matter load. *Microbial Biotechnology,* **14**: 726-736.

Wang, X., Yu, S., Zhou, L. and Fu, S. (2016). Soil microbial characteristics and the influencing factors in subtropical forests. *Acta. Ecol. Sin.,* **36**: 8-15.

Wang, Y., Zhang, J., Zhang, Z. and Jia, L. (2016). Impact of tillage erosion on water erosion in a hilly landscape. *Sci. Total Environ.,* **551**: 522-532.

Warren, R., Vanderwal, J., Price, J., Welbergen, J.A., Atkinson, I., Ramirez-Villegas, J., Osborn, T.J., Jarvis, A., Shoo, L.P. and Williams, S.E. (2013). Quantifying the benefit of early climate change mitigation in avoiding biodiversity loss. *Nat. Clim. Chan.,* **3**: 678.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bact.*, **173**: 697-703.

Widawati, S. (2018). The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination and Seedling Growth of Sorghum bicolor L. Moench. **In:** *Proceeding The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination and Seedling Growth of Sorghum bicolor L. Moench*, pp. 012022.

Wielbo, J., Marek-Kozaczuk, M., Kubik-Komar, A. and Skorupska, A. (2007). Increased metabolic potential of Rhizobium spp. is associated with bacterial competitiveness. *Can. J. Microbiol.,* **53**: 957-967.

Wiseman, P.E., Day, S.D. and Harris, J.R. (2012). Organic amendment effects on soil carbon and microbial biomass in the root zone of three landscape tree species. *Arboriculture & Urban Forestry,* **38**: 262-276.

Withers, P.J., Ulén, B., Stamm, C. and Bechmann, M. (2003). Incidental phosphorus losses–are they significant and can they be predicted? *J. Plant Nutr. Soil Sci.,* **166**: 459-468.

Wong, M. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere,* **50**: 775-780.

World Bank (2001) African Development Indicators. Washington. Pp. 67-101.

Wu, F., Wan, J.H.C., Wu, S. and Wong, M. (2012). Effects of earthworms and plant growth–promoting rhizobacteria (PGPR) on availability of nitrogen, phosphorus, and potassium in soil. *J. Plant Nutr. Soil Sci.,* **175**: 423-433.

Wu, H., Lai, C., Zeng, G., Liang, J., Chen, J., Xu, J., Dai, J., Li, X., Liu, J. and Chen, M. (2017). The interactions of composting and biochar and their implications for soil amendment and pollution remediation: a review. *Critical reviews in biotechnology,* **37**: 754-764.

Wu, Y., Zhang, J. and Guo, X. (2017). An indigenous soil bacterium facilitates the mitigation of rocky desertification in carbonate mining areas. *Land Degrad. Dev.,* **28**: 2222-2233.

Xavier, A., Lima, E., Oliveira, A., Cardoso, L., Santos, J., Cangussu, C., Leite, L., Quirino, M., Oliveira, D. and Xavier, M. (2017). Genetic diversity of Bacillus sp producers of amylase isolated from the soil. *Gen. Mol. Res.,* **16**.

Xie, Z., Tu, S., Shah, F., Xu, C., Chen, J., Han, D., Liu, G., Li, H., Muhammad, I. and Cao, W. (2016). Substitution of fertilizer-N by green manure improves the sustainability of yield in double-rice cropping system in south China. *Field Crops Res.,* **188**: 142-149.

Xing, Y.-X., Wei, C.-Y., Mo, Y., Yang, L.-T., Huang, S.-L. and Li, Y.-R. (2016). Nitrogen-fixing and plant growth-promoting ability of two endophytic bacterial strains isolated from sugarcane stalks. *Sugar Tech,* **18**: 373-379.

Xu, G. and Zheng, H. (1986). Handbook of analysis methods of soil microbiology. *Agricultural, Beijing, China*: 102-119.

Yadav, J., Verma, J.P. and Tiwari, K.N. (2010). Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (Cicer arietinum L.) under in vitro conditions. **In:** *Proceeding Effect of plant growth promoting rhizobacteria on seed germination and plant growth chickpea (Cicer arietinum L.) under in vitro conditions,* pp. 15-18.

Yang, J., Kloepper, J.W. and Ryu, C.-M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.,* **14**: 1-4.

Yang, L., Bian, X., Yang, R., Zhou, C. and Tang, B. Assessment of Organic Amendments for Improving Coastal Saline Soil. *Land Degradation & Development*.

Yang, L., Bian, X., Yang, R., Zhou, C. and Tang, B. (2018). Assessment of organic amendments for improving coastal saline soil. *Land Degrad. Dev.,* **29**: 3204-3211.

Yao, L., Wu, Z., Zheng, Y., Kaleem, I. and Li, C. (2010). Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton. *Eur. J. Soil Biol.,* **46**: 49-54.

Yazdani, M., Bahmanyar, M.A., Pirdashti, H. and Esmaili, M.A. (2009). Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (Zea mays L.). *World Acad. Sci. Eng. Technol.,* **49**: 90-92.

Yesuf, M., Di Falco, S., Deressa, T., Ringler, C. and Kohlin, G. (2008). ***The impact of climate change and adaptation on food production in low-income countries: evidence from the Nile Basin, Ethiopia***. Intl Food Policy Res Inst, Addis Ababa, pp. 24.

Yitbarek Tibebe, Belliethathan, S. and Stringer, L. (2012). The onsite cost of gully erosion and cost-benefit of gully rehabilitation: A case study in Ethiopia. *Land Degrad. Dev.,* **23**: 157-166.

Yoke-Kqueen, C., Teck-Ee, K., Son, R., Yoshitsugu, N. and Mitsuaki, N. (2013). Molecular characterisation of Vibrio parahaemolyticus carrying tdh and trh genes using ERIC-, RAPD- and BOX-PCR on local Malaysia bloody clam and Lala. *Int. Food Res. J.,* **20**: 3299.

Zahid, M. (2015). Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (Zea mays L.). *Front. Microbiol.,* **6**: 207.

Zahran, H.H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev.,* **63**: 968-989.

Zajic, J. and Supplisson, B. (1972). Emulsification and degradation of "Bunker C" fuel oil by microorganisms. *Biotechnol. Bioeng.,* **14**: 331-343.

Zhang, J., Xin, Y. and Zhao, Y. (2019). Diversity and Functional Potential of Soil Bacterial Communities in Different Types of Farmland Shelterbelts in Mid-Western Heilongjiang, China. *Forests,* **10**: 1115.

Zhang, N., Yang, D., Kendall, J.R., Borriss, R., Druzhinina, I.S., Kubicek, C.P., Shen, Q. and Zhang, R. (2016). Comparative genomic analysis of Bacillus amyloliquefaciens and Bacillus subtilis reveals evolutional traits for adaptation to plant-associated habitats. *Front. Microbiol.,* **7**: 2039.

Zimmerman, A.R. (2010). Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environ. Sci. Technol.,* **44**: 1295-1301.

Zingore, S., Delve, R.J., Nyamangara, J. and Giller, K.E. (2008). Multiple benefits of manure: the key to maintenance of soil fertility and restoration of depleted sandy soils on African smallholder farms. *Nutr. Cycl. Agroecosystems,* **80**: 267-282.

Zulueta-Rodríguez, R., Hernández-Montiel, L., Murillo-Amador, B., Rueda-Puente, E., Capistrán, L., Troyo-Diéguez, E. and Córdoba-Matson, M. (2015). Effect of hydropriming and biopriming on seed germination and growth of two Mexican fir tree species in danger of extinction. *Forests,* **6**: 3109-3122.

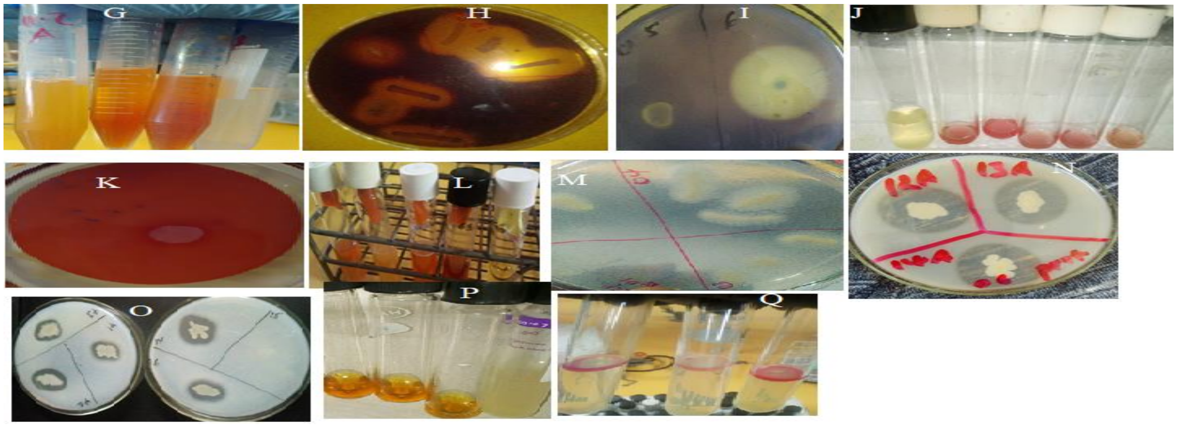
List of Appendices

Appendix 1. Partial view of study site, OAs collection and applications

Appendix 2. Partial view of the fenced off land and land ploughing activity

Appendix 3. Bacterial isolation and purifications

Appendix 4. Some *in vitro* drought stress (DS) associated traits produced by potential PGPR. R = Biofilm production using Congo red agar, T and U= Biofilm formations on glass and plastic test



Appendix 5. Some sample *in vitro* plant growth promoting traits. G= Ammonia production, H= CMC activity, I= Chitinase activity, J and Q= IAA production, K and L= HCN production using plate and tube methods, M= Lipase activity, N= Protease activity, O=Phosphate solubilization, P= Siderophore production

Appendix 6. DNA extraction, gel mix and loading and gel electrophoresis of some rhizobacterial

Appendix 7. Greenhouse activities on the performance of cover crops upon OAs, the growth of acacia with rhizobacterial inoculations for water stress enhancement test.

Appendix 8. Partial 16S rRNA genes sequencing of rhizobacteria, accession numbers, closest NCBI match, and % similarity

Isolate	Gene Bank accession no.	Most closely related organisms*				
		Species	Accession description	% Gene identity	% Query coverage	E -value
BS-22	MN005961	*Bacillus anthracis*	CP033795.1	100	100	0.0
BS-29	MN005962	*Bacillus cereus*	AY853168.1	100	99	0.0
BS-32	MN005963	*Bacillus anthracis*	CP033795.1	100	99	0.0
BS-37	MN005964	*Bacillus cereus*	AJ539175.1	99	99	0.0
BS-40	MN005965	*Bacillus cereus*	AY853168.1	99	100	0.0
BS-45	MN005966	*Bacillus thuringiensis*	KX641526.1	99	100	0.0
BS-47	MN005967	*Bacillus thuringiensis*	KX641526.2	99	100	0.0
PS-4	MN005968	*Enterococcus gallinarum*	CP033740.1	99	100	0.0
PS-5	MN005969	*Enterococcus gallinarum*	CP033740.1	99	100	0.0
PS-8	MN005970	*Enterococcus gallinarum*	CP033740.1	99	100	0.0
PS-9	MN005971	*Enterococcus gallinarum*	CP033740.1	99	100	0.0
PS-11	MN005972	*Enterococcus gallinarum*	JF915769.1	92	99	0.0
PS-16	MN005973	*Enterococcus gallinarum*	JF915769.1	99	99	0.0
BS-51	MN005974	*Paenibacillus polymyxa*	CP006872.1	98	100	0.0
BS-30	MN005975	*Paenibacillus odorifer*	CP009281.1	100	100	0.0
FB-50	MN005976	*Paenibacillus polymyxa*	CP025957.1	100	100	0.0
RS-79	MN005977	*Agrobacterium tumefaciens*	CP033032.1	99	100	0.0
RS-58	MN005978	*Ochrobactrum intermedium*	KC146415.1	100	99	0.0
RS-60	MN005979	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-61	MN005980	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-63	MN005981	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-66	MN005982	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-67	MN005983	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-68	MN005984	*Ochrobactrum intermedium*	AJ242582.2	99	100	0.0
RS-69	MN005985	*Ochrobactrum intermedium*	KC146415.1	99	99	0.0
RS-70	MN005986	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-71	MN005987	*Ochrobactrum intermedium*	KC146415.1	99	100	0.0
RS-72	MN005988	*Ochrobactrum intermedium*	KC146415.1	100	100	0.0
RS-74	MN005989	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
RS-76	MN005990	*Ochrobactrum anthropi*	KC146415.2	100	100	0.0
RS-77	MN005991	*Ochrobactrum intermedium*	AJ242582.2	99	99	0.0
BS-27	MN005992	*Acinetobacter calcoaceticus*	KC257031.1	99	99	0.0
BS-19	MN005993	*Pseudomonas putida*	CP025262.1	99	99	0.0
BS-21	MN005994	*Pseudomonas fulva*	CP014025.1	100	99	0.0
BS-23	MN005995	*Pseudomonas fulva*	CP014025.1	100	100	0.0

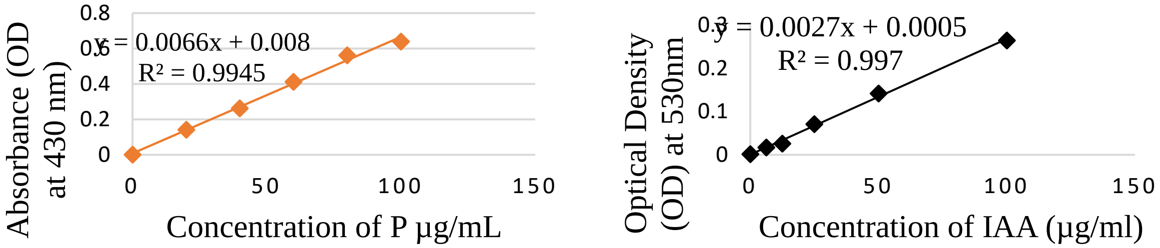
Table 5. Continued.

Isolate	Gene Bank accession no.	Most closely related organisms*				
		Species	Accession description	% Gene identity	% Query coverage	E -value
BS-24	MN005996	*Pseudomonas fulva*	CP014025.1	100	00	0.0
BS-26	MN005997	*Pseudomonas plecoglossicida*	MF281997.1	99	100	0.0
BS-28	MN005998	*Pseudomonas fulva*	CP014025.1	99	100	0.0
BS-31	MN005999	*Pseudomonas fulva*	CP014025.1	100	99	0.0
BS-34	MN006000	*Pseudomonas fulva*	CP014025.1	99	100	0.0
BS-36	MN006001	*Pseudomonas fulva*	CP014025.1	99	100	0.0
BS-39	MN006002	*Pseudomonas fulva*	CP014025.1	100	100	0.0
BS-41	MN006003	*Pseudomonas fulva*	CP014025.1	100	100	0.0
BS-43	MN006004	*Pseudomonas fulva*	CP014025.1	99	100	0.0
BS-44	MN006005	*Pseudomonas fulva*	CP014025.1	99	100	0.0
BS-53	MN006006	*Pseudomonas fulva*	CP014025.1	99	100	0.0
RS-75	MN006007	*Pseudomonas protegens*	MK182884.1	99	100	0.0
FB-49	MN006008	*Pseudomonas fluorescens*	KY228953.1	100	100	0.0
PS-1	MN006009	*Klebsiella michiganensis*	CP033824.1	99	99	0.0
PS-2	MN006010	*Klebsiella michiganensis*	CP033824.1	99	100	0.0
PS-3	MN006011	*Klebsiella oxytoca*	CP033824.2	99	99	0.0
BS-46	MN006012	*Morganella morganii*	CP032295.1	99	99	0.0
PS-6	MN006013	*Morganella morganii*	CP032295.1	99	99	0.0
PS-10	MN006014	*Morganella morganii*	CP032295.1	99	100	0.0
PS-12	MN006015	*Morganella morganii*	CP032295.1	99	99	0.0
PS-13	MN006016	*Morganella morganii*	HQ774675.1	99	100	0.0
PS-14	MN006017	*Morganella morganii*	CP032295.1	100	100	0.0
PS-15	MN006018	*Morganella morganii*	CP032295.1	99	99	0.0
PS-18	MN006019	*Morganella morganii*	CP032295.1	100	100	0.0
RS-78	MN006020	*Morganella morganii*	CP032295.1	99	100	0.0
BS-35	MN006021	*Pantoea vagans*	CP014129.2	99	99	0.0
BS-38	MN006022	*Pantoea vagans*	CP014129.2	99	99	0.0
BS-20	MN006023	*Serratia grimesii*	CP033162.1	99	100	0.0
BS-42	MN006024	*Serratia grimesii*	MG972923.1	100	100	0.0
PS-54	MN006025	*Serratia fonticola*	LR134492.1	99	100	0.0
RS-65	MN006026	*Serratia marcescens*	CP021164.1	99	99	0.0
RS-73	MN006027	*Serratia grimesii*	CP033162.1	100	100	0.0
RS- 57	MN006028	*Serratia grimesii*	CP033162.1	99	100	0.0
RS- 62	MN006029	*Serratia grimesii*	CP033162.1	99	100	0.0
RS-64	MN0060230	*Serratia fonticola*	LR134492.1	99	99	0.0

* Data obtained after BLAST analysis from NCBI database

Appendix 9. Examples of some nursery tree for transplantation, prunus (left) and acacia (right)

Appendix 10. The application of cover crop on organically amended (left) and nonamended plots (right)



Appendix 11. Standard curve for the amount of P-solubilized and IAA produced by each potential PGPR Concentration of P and IAA X-axis vs OD value at 530 nm on Y- axis) respectively.

Appendix 12. Burrow preparation, multipurpose tree transplanting, rhizobacterial inoculation and cultivation

Growth of Apple following OAs and BI on degraded soil

Control plot

Growth of acacia f ollowing OAs and BI application on degraded soil

Control plot

Growth of prunus following AOs and Bi application on degraded soil

Control plot

Appendix 13. The growth performance and establishment of apple, acacia and prunus on degraded land following OAs and microbial inoculations.