QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

LINKING SOIL CHEMICAL PARAMETERS AND ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN QATAR

BY

SAKEENAH BINTE ADENAN

A Thesis Submitted to

the College of Arts and Sciences

in Partial Fulfillment of the Requirements for the Degree of

Masters of Science in Environmental Sciences

June 2020

COMMITTEE PAGE

The members of the Committee approve the Thesis of
Sakeenah Binte Adenan defended on 17/08/2020.

Juha Mikael Alatalo
Thesis/Dissertation Supervisor

Talaat Abdelfattah Ahm Youssef
Thesis Co-Supervisor

Roda Fahad Al-Thani
Committee Member

Amjad Mahmoud Ahmad Shraim
Committee Member

Samir Jaoua
Committee Member

Approved:

Ibrahim AlKaabi, Dean, College of Arts and Sciences

ABSTRACT

ADENAN, SAKEENAH, B., Masters : June : 2020, Master of Science in Environmental Sciences

Title: Linking Soil Chemical Parameters and Arbuscular Mycorrhizal Fungal Communities in Qatar

Supervisor of Thesis: Juha, M, Alatalo.

Much of Qatar's habitats are characterized by a hyper-arid climate, low rainfall and scarce soil nutrients; all of which combine to create a stress-induced environment for fungal development. Agricultural productivity and soil fertility in turn, are highly dependent on the diversity of soil microbiota. The soil environment is a heterogeneous habitat shaped by various components including soil chemical properties and habitat-types i.e. wetlands, barren land and grasslands. Arbuscular mycorrhizal fungi (AMF) are one of the key components vital for ecological processes in stress-induced systems as the complex hyphae systems of AMF assemblages permit sufficient nutrient exchange in mycorrhiza symbionts. However, much of the diversity and community composition of AMF in Qatar are poorly studied. In this study, Illumina sequencing by MiSeq platform was used to identify and explore the diversity and composition of AMF communities. Our results revealed 127 virtual taxa (VTs) from eight AMF families, 87 of which were identified as species from *Glomeraceae* family; while indicator analysis showed that *Glomeraceae* species served as the indicator species in 15 sites with an indicator index value of 13.33 (indicator value > 0.25).

The chemical properties investigated in this study accounted for 76% of AMF variability. Initial hypothesis stated that (1) AMF abundance and diversity is positively influenced by increasing quantities of macronutrients (that is, Ca, Mg, P, K, NO_2^- and NO_3^-); while this assumption was true for P and K, results in this study showed that the effect of all other nutrients (Ca, Mg, NO_2^- and NO_3^-) on AMF was not statistically significant at 95% confidence interval. (2) Regardless of its quantity, the presence of Cd and Pb within the soil environment would negatively affect the occurrence and abundance of AMF species due to the adverse toxicity induced by these heavy metals. (3) An increase in soil salinity and pH will negatively influence AMF communities as soil conditions reaches extremities that restrict soil fungi to flourish. This assumption was proven not to be true as the significance of these soil components (pH and salinity) on AMF abundance was not statistically significant. AMF diversity found in sites that were largely dominated by coastal shores, mangroves and wetlands (i.e. regions with high salinity, ~5.43 ± 0.07 psu and pH,~9.07 ± 0.06); were among the lowest. It is likely that present AMF in Qatar had been exposed to high salinity levels through their evolutionary history in the region. This prolonged exposure favoured AMF with a natural resistance to salinity through natural selection. Extreme soil conditions reduced the overall abundance and diversity of fungal species but rather, supported the colonization of more halotolerant fungi.

Our hypothesis pertaining the heavy metals, Cd and Pb, was proven to be incorrect. Although regression analysis indicated a positive correlation for both Cd and Pb on AMF, the effect was statistically significant for Cd only. Despite that, this study lacked sufficient data addressing the metabolic response of AMF in Cd-contaminated soils. Further research should therefore, focus on the metabolic pathways of AMF assemblages associated with heavy metal toxicity and salt stress. These findings

provide baseline information for researchers to delve deeper into the functional profiles of AMF assemblages and explore its role in stress-induced environments such as Qatar.

DEDICATION

This thesis is dedicated to my parents, Adenan Mohamed

and Siti Aishah Salim.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation and gratitude towards my supervisors, Dr. Juha Mikael Alatalo and Dr. Talaat Abdelfattah, without whom, successful completion and smooth-sailing of my research work would not have been possible. For their unwavering support, continuous optimism and comprehensive advice during the course of my research, I am truly grateful. It has been a great honour working under their guidance.

I humbly extend my gratitude towards my committee members and professors, Dr. Amjad Shraim, Dr. Samir Jaoua and Dr. Roda Al-Thani for their assistance and continuous encouragement presented throughout the course of my study at Qatar University.

I would also like to acknowledge the staff at Environmental Science Centre for allowing me to use their facilities and faculty members from the Department of Biological and Environmental Science for providing a supportive and pleasant study environment for all students. My appreciation also goes to Dr. Jane Oja, without whom, this research journey would not have been easy.

My final gratitude extends to my family and friends for their moral support, all of whom have made my journey as a student at Qatar University more enjoyable.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PCA	Principle Component Analysis
PC	Principle Component
ANOVA	Analysis of Variance
df	Degrees of freedom
RDA	Redundancy Analysis
AHC	Agglomerative Hierarchical Clustering
AMF	Arbuscular Mycorrhizal Fungi
EcM	Ectomycorrhiza
BLAST+	Basic Local Alignment Search Tool
INSDC	International Nucleotide Sequence Database Collection
HTS	High-throughput Sequencing
NGS	Next Generation Sequencing
ITS	Internal Transcribed Spacer
LSU	Large Subunit
SSU	Small Subunit
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
bp	Base pairs
VT	Virtual Taxa
MID	Multiplex Identifier
PCR	Polymerase Chain Reaction

OTU	Operational Taxonomy Unit
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
CRM	Certified Reference Material
EPA	Environmental Protection Agency
DL	Detection limit
CEC	Cation Exchange Capacity
NEDD	N-1-apthylenediamine dihydrochloride
KCl	Potassium chloride
EC	Electrical Conductivity
TDS	Total Dissolved Solids
PTFE	Polytetrafluoroethylene
OC	Organic Carbon
TC	Total Carbon
TN	Total Nitrogen

CHAPTER 1: INTRODUCTION

With the rapid increase in global population, the demand for ecosystem goods and services are continuously rising. Global drylands support a significant proportion of the global population by providing potential agricultural fields and ecosystem provisions essential for human sustenance. The development of such arid lands is, therefore, imperative in order to obtain sustainable development. Given the vast expanse of Qatar's dryland ecosystems, an understanding on the relationship between soil fungal diversity and heterogeneity of Qatar's habitat is a key aspect in maintaining the overall stability and fertility of arid lands. Fungal diversity changes along environmental gradients affected by components such as soil nutrients, vegetation cover, moisture, pH and salinity. However, scientific data on the impact of these chemical parameters and environmental aspects on the diversity and composition of soil microbiota in Qatar remains poorly known due to the lack of systematic assessments carried out in the region.

Arbuscular mycorrhizal fungi (AMF) present within Qatari soil was identified at the genus level through the use high-throughput sequencing technology. Consequently, the relationship between fungal diversity and land heterogeneity in Qatar was assessed, while the chemical properties of soils collected from various sampled locations were analysed to determine a potential correlation between AMF diversity and abundance with its corresponding chemical components including total carbon (TC) and nitrogen (TN) content, concentration of macronutrients (i.e. nitrite, nitrate, calcium, magnesium, phosphorous and potassium), present heavy metals (i.e. cadmium and lead), soil salinity, electrical conductivity (EC) and pH. Molecular identification of AMF involved several processes including collection of soil samples from different study sites across Qatar, extraction of environmental genomic DNA, bioinformatics and

data processing. Identification of AMF families in this present study was carried out using a culture-independent technique. Illumina MiSeq technology was employed for the purpose of fungal identification and subsequently, quantification of the fungal diversity and abundance present within Qatar's hyper-arid environment. The distribution of AMF families between different land-types and AMF diversity was assessed in relation to the chemical characteristics and biological (that is, vegetation coverage and present plant species) factors of the corresponding study sites.

This study aims to evaluate potential soil properties and chemical parameters that may affect the distribution and diversity of mycorrhizal fungi. Based on past research and ecological studies (Kasel et al., 2008; Trivedi et al., 2016; Vyas & Gupta, 2014; Xue et al., 2018), we hypothesize that differences in the chemical properties of soils between locations, as a result of different land uses and vegetation cover, will influence the fungal diversity, abundance and composition. Specifically, we hypothesize that; (1) an increase in soil salinity and pH will negatively influence AMF communities by reducing the total abundance and diversity of AMF species as soil conditions reaches extremities that restrict soil fungi to flourish, (2) AMF abundance and diversity will be positively associated with increasing quantities of macronutrients (that is, Ca, Mg, P, K, NO_2^- and NO_3^-) and (3) given the adverse toxicity of heavy metals on microbial communities, the presence of Cd and Pb within the soil environment will produce a significant negative effect on the occurrence and abundance of AMF species, regardless if these heavy metals were present in small quantities.

CHAPTER 2: LITERATURE REVIEW

2.1 Dryland Ecosystems and Habitat

Including Qatar's desert lands, the global desert regions make up approximately one-third of Earth's surface and therefore, play a significant role in the global carbon cycle (Neilson et al., 2017). Dryland ecosystems are depicted to be highly unstable habitats. However, given the severe environmental conditions in several drylands, research has proven these unstable ecosystems to be extremely resilient. The ability of dryland ecosystems to maintain its functional stability by adjusting to environmental drivers justifies its resilient nature. For instance, spatial re-establishment of plantations in African drylands since 1980 justifies the resilience of many dryland ecosystems (United Nations, 2011). However, the capacity for drylands to acclimatize according to its environmental conditions is dependent on the presence of certain essential components within the habitat including soil nutrients and soil microbiota as a means of sustaining the integrity of dryland ecosystems (Laban et al., 2018). Due to the vast expanse of drylands around the world, the biodiversity and biological activity driven by such ecosystems serve to be an important integral in the global community. At present, drylands are home to a large variety of endemic species including plants, living organisms and fungal species; some of which exhibit unique adaptations to the harsh conditions presented by drylands (Laban et al., 2018; Neilson et al., 2017).

In addition to the various ecosystem services and goods produced from dryland systems, a significant proportion of the global food production is also provided by these fragile habitats. However, majority of drylands around the world are at risk of degradation as a result of human intervention of invasive species, overexploitation and anthropogenic activities (Reynolds et al., 2007; United Nations, 2011). At present, the need to conserve the biodiversity in drylands has become increasingly imperative due

to the substantial coverage of the global dryland habitats. Moreover, over half the global population are dependent on the services and provisions derived from these habitats such as raw materials and pharmaceutical supplies while offering aesthetic and cultural benefits for many dryland regions (United Nations, 2011). The total global contribution of soil biodiversity towards ecosystem functioning and services is estimated to be between USD 1.5 and 13 trillion per year (Laban et al., 2018). Such commodities and ecosystem goods offered from drylands around the world is often a result of their functional biodiversity along with the processes and environmental conditions through which nature can sustain human life. The current protected habitats across the world covers approximately 9% or 5.4 million km^2 of global drylands (Laban et al., 2018). Compared to other protected environments, drylands remain the least protected habitat in the world, likely due to the lack of management and understanding of its ecological importance.

2.2 Soil Properties as Indicators of Soil Health

2.2.1 Chemical Properties

The structural and nutritional support of the soil environment is an essential component for agricultural sustenance and ecosystem functioning. Soil environments can directly and indirectly impact agricultural productivity, quality of water and the global climate; acting as the very regulator of nutrient cycling and water flow (Delgado-Baquerizo et al., 2015; Delgado & Gómez, 2016). A stable soil environment is one that exhibit characteristics that enhances the supply and storage of nutrients and water to growing crops and vegetation. The soil chemistry, however, is largely influenced by interaction between insoluble components like silica and its water phase (Delgado & Gómez, 2016; Villalobos et al., 2016). Soil chemistry forms a fundamental basis to soil fertility and agricultural productivity. In most cases, the soil chemistry of any given

area directly affects the physical condition of the surrounding area, such as that presented by sodic soils with high sodium exchange capacity. Additionally, the soil environment is also inhabited by a complex fauna of microorganisms driving various biochemical processes, which in turn, also affects the physical and chemical properties and ultimately, agricultural productivity (Tian et al., 2017).

2.2.1.1 Soil pH

Soil pH is often presented as a measurement of retained protons (H^+) within the soil aggregates (Wang et al., 2017). Determination of soil pH is generally indicated as the pH of soil solution in water or salt (generally 0.1M $CaCl_2$). According to Delgado & Gómez (2016), the degree of soil alkalinity or acidity is a relevant indicator of soil health as it directly affects several biological and physicochemical processes. In most cases, the problem of acidic soils is overcome by enhancing the base saturation of the soil via processes like liming (Delgado & Gómez, 2016). Basic soils in turn, are the result of present basic elements or buffering compounds such as carbonates. Alternatively, calcareous soils are those that contain a significant concentration of calcium carbonate, with a buffering pH of approximately 8.5. However, the presence of other carbonates like magnesium or sodium can increase the soil pH to well above 8.5 (McHugh et al., 2017; Villalobos et al., 2016). Alterations of the buffering capacity in calcareous soils has proven to be a more challenging process. Agricultural uses of this soil type are therefore, limited as nutrient uptake and plant growth is restricted by the nature of the soil.

Certain soil characteristics including mineral availability, physical properties and soil microbiota are influenced by soil pH (Oehl et al., 2010; Tian et al., 2017). Soils exhibiting low pH are often associated with base nutrient deficiency such as calcium (Ca), magnesium (Mg) and potassium (K), thereby reducing its bioavailability (Bansal,

2018). Additionally, Delgado & Gómez (2016) reported that solubility of phosphorous (P) and molybdenum (Mo) decreased at low pH levels and in turn, also reduced its bioavailability. Alternatively, their study reported increasing aluminium (Al) concentrations (typically at pH less than 5.5); resulting in an increase in aluminium toxicity of the soil environment (Wu et al., 2018). At significantly low pH, the concentration of trace elements like aluminium (Al), iron (Fe) and manganese (Mn) may be high enough to cause soil toxicity. On the other hand, higher pH values could decrease the solubility of such trace metals; including essential elements (e.g. iron, zinc, copper and manganese) needed for plant growth despite its potential toxicity at high concentrations (Bansal, 2018). Due to reduced solubility of their elements, basic soils are generally deficient in trace metals such as iron, thereby resulting in iron chlorosis (Delgado & Gómez, 2016).

In terms of the biological and physical components, extreme pH levels have been linked with reduced microbial activity within the soil environment (Delgado & Gómez, 2016; Tian et al., 2017). Such microbial processes like organic matter decomposition, soil nitrification and biological nitrogen fixation may be hindered as the microbial consortium is altered under acidic soil conditions (Hannachi et al., 2015). Additionally, acidic soils have also been linked with low calcium (Ca) concentrations and an increased diffusion of aluminium colloids (Delgado & Gómez, 2016). As a result, acidic soils are generally associated with poor physical soil conditions as the structural support and permeability of soil environments are often deterred (Delgado & Gómez, 2016; Trivedi et al., 2016).

2.2.1.2 Ions Retention

The formation of solid composites as a result of the interaction between two chemical components may be described as the precipitation process, e.g. formation of

calcium phosphate crystals upon addition of phosphorous fertilizer (Hannachi et al., 2015). Alternatively, adsorption of chemical components is generally described as the accumulation of chemical sorbate onto existing solid surfaces (sorbent). Despite differences in chemical composition through precipitation or adsorption, both processes (that is, adsorption and precipitation) have the capacity to retain essential ions within the soil (Delgado & Gómez, 2016). Precipitated and adsorbed compounds exist in equilibrium with the soil solution. While adsorption is typically the result of sorbate-specific chemical retention onto hydroxylated surfaces, it may also be the consequence of non-sorbate specific electrostatic attraction (Aliasgharzadeh et al., 2001; Tian et al., 2017). Associations between mineral and organic sorbent surfaces may vary between environments; ion retention within the soil environment is therefore, dependent on the charge between the chemical component and medium (Wang et al., 2015). However, specific electrostatic attractions generally arise from isomorphic compounds within clay soil minerals. On the other hand, variable charge attractions are pH dependent and are often the result of incomplete bonds of minerals and organic matter (Delgado & Gómez, 2016).

2.2.1.3 Soil Salinity

Soil salinity may be defined as the concentration of soluble salts within the soil environment. Highly saline soils will generally comprise of salt concentrations high enough to negatively affect the development and growth of most cultivated plant crops (Delgado & Gómez, 2016; Howard, 2010). The classification of soil salinity and determination of its negative impacts on fungal growth and occurrence is based on its level of tolerance. Given their adaptation abilities, these halotolerant fungi are often found in regions of harsh environmental conditions including high salinity and extreme drought. According to Villalobos et al. (2016), ample evidence have indicated variation

in salinity associated with different land types. One study investigating fungal tolerance to soil salinity found that certain fungal species such as *Penicillium* and *Aspergillus* have the ability to resist and sporulate in highly-saline soils (Tresner & Hayes, 1971). While some studies (Al-Tamie, 2014; Krasensky & Jonak, 2012) reported the impediment of metabolic reactions in highly saline environments, more recent investigations demonstrated the adaptability of soil fungi to flourish in saline concentrations up to 200 g/l (Rouphael et al., 2015).

Much of the terrain in the Middle East, including Qatar, consist of low-lying sabkhas and saltmarshes. These habitats are often subjected to an influx of water, either from coastal regions or rainfall, thus, leading to flood lands. Due to the arid climate associated with most desert environments, the water influx in low-lying sabkhas evaporate to form a substratum of salt (Norton et al., 2009), thereby allowing the growth of halophytes and proliferation of halotolerant fungal assemblages. On the other hand, continuous drying of these soil environments enhances the salt content in these areas and ultimately, intensify soil salinity to a degree that is well over the tolerance threshold (Baumann & Marschner, 2013). Combined with the lack of precipitation, these physiological challenges could potentially threaten the survival and functioning of fungal assemblages. Moreover, Oren (2008) reported that microbial responses toward drought and salinity challenges can be energetically draining as ionic concentration gradients between cell membranes must be maintained. High salt content in soils causes osmotic stress on the survival of soil microbes (Baumann & Marschner, 2013; Yan et al., 2015), thus hindering key ecological functions. However, it can be assumed that the stressful nature brought by drought and salinity would cause a shift in the distribution and diversity of fungal communities as these stressors combined will favour the survival of fungal species that are better adapted or more tolerant.

2.2.2 Biological Properties

The soil environment is home to a complex web of organisms that directly influences soil evolution and its consequent physical and chemical characteristics (Van Der Heijden et al., 2008). For instance, increasing microbial activity in any given soil environment reduces the organic matter as a result of mineralization (Delgado & Gómez, 2016). It is widely known that soil biological properties exhibits a strong interconnection with other physical and chemical components including soil aeration, pH and organic matter content, and therefore, directly influences essential biochemical processes like carbon and nutrient cycling. Given the sensitivity of microbial matter, minor changes in soil properties and management may significantly affect its biological composition. For instance, Delgado & Gómez (2016) reported an increase in soil microbial activity associated with improved drainage and soil organic alterations. The efficacy of biological properties within a given soil environment have been demonstrated in numerous studies (Frac et al., 2018; Neilson et al., 2017; Wang et al., 2017) as it has been regarded as a direct indicator of environmental quality.

Soil organic matter is one of the key contributors influencing the biological composition in soil environments as it forms the largest carbon source for soil microbiota (Van Der Heijden et al., 2008). Combined with the quantity of available organic matter, the type of organic compound also influences the diversity and activity of soil microorganisms i.e. microbial activity is greatly enhanced upon the addition of organic crop residues as it is easily mineralized by soil microbes (Wu et al., 2018). Alternatively, more stable forms of soil consisting of humic and fulvic compounds, such as those present in temperate regions, does not constitute a readily available source of carbon for existing soil microbes (Delgado & Gómez, 2016).

Combined with the quantity of organic compounds, the type of organic components present also plays a vital role in soil biological activity; incorporation of fresh manure and organic residue enhances microbial activity. Despite the high organic content found in humic soils, bioavailability of the carbon source is less suited for existing soil microbiota since the organic matter exists as a stable entity, which justifies the extended half-life presented by organic matter in such stable soil systems (typically >1000 years) (Xue et al., 2018). Stable organic compounds are, therefore, not considered to be major contributors to soil microbial activity. It does, however, contribute to a stable carbon reservoir that in turn, forms a major constituent to the global carbon cycle; partially neutralizing the effects of increasing carbon emissions into the atmosphere (Delgado & Gómez, 2016).

2.3 Chemical Parameters and Land Heterogeneity

Soils are generally regarded as complex ecosystems, consisting of various mineral compounds and organic matter that interact with soil microorganisms to create a diverse community of species; with each individual species playing a role to perform essential ecosystem functions (Oehl et al., 2010). Combined with the chemical properties of the soil and environmental conditions of the given habitat, the mineral composition and diversity of soil fungi determines the overall structural stability and fertility of the habitat (Tian et al., 2017). Soil microbiota in turn, is dependent on several environmental components such as climate, relative humidity, temperature, salinity as well as decomposed plant material. Laban et al. (2018) suggested that diversity of soil microbes is likely to increase with increasing humidity. This assumption is based on the capacity of microorganisms to flourish when placed under optimum conditions. The extent of moisture retention in turn, is largely determined by the soil structure and ratio of organic matter to organic carbon. One study suggests that a reduction of soil organic

matter by 1g results in a decrease of soil moisture content by $1x10^{10}$g (Laban et al., 2018). A diverse community of soil fungi is essential to support agricultural productivity and plant biodiversity (Tian et al., 2017), even more so in desert areas where soil systems are generally fragile habitats, susceptible to environmental changes. Soil fungal diversity is a major determinant of the agricultural success (McHugh et al., 2017), while also serving as the central foundation for sustainable agriculture and resilience towards climate change (Mandal & Sathyaseelan, 2012).

Microbial community in dryland soils is one that is subjected to external conditions, environmental characteristics and structure of the soil itself. For instance, the genetic diversity and abundance of bacterial species in Mezquital Valley, Mexico is highly influenced by irrigation practices (Lüneberg et al., 2018). Results from Lüneberg et al.'s study (2018) indicated that the genetic abundance of bacterial species involved in carbon, nitrogen and phosphorous cycles are more susceptible to environmental changes in freshwater irrigated soils, whereas the genetic abundance found in wastewater irrigated soils are more resilient towards environmental changes. Wastewater irrigated soils exhibited higher microbial and enzymatic activity due to the supply of nutrients and organic matter from the wastewater. In this regard, the principle that soil diversity is driven by the chemical properties is justified. Such properties like soil pH, moisture, nutrient and carbon content are generally influenced by the environmental components of the habitat like land use, vegetation cover, climate and irrigation. However, other studies presented results that opposed such hypothesis, suggesting that microbial species in dryland soil are primarily determined by moisture content and pH, while the influence of organic carbon content on microbial diversity is relatively insignificant (Delgado-Baquerizo et al., 2015; McHugh et al., 2017; Xue et al., 2018). Moreover, the transition of dryland ecosystems into agricultural plantations

could potentially shift the genetic variation of fungi communities. Transformation of drylands into plantation fields has been shown to shift the genetic diversity of soil microbes. According to Lüneberg et al. (2018), conversion of hyper-arid drylands into agricultural fields resulted in an apparent increase in the soil bacterial diversity, while the opposite was shown semi-arid regions (that is, a reduction in bacterial diversity in agriculturally-converted drylands). Variability in soil chemical parameters as a result of land use, transformation and human-based activities of natural dryland ecosystems will likely influence the diversity, composition and abundance of soil fungi.

Xue et al. (2018) demonstrated the importance of belowground microbial communities by their role as ecosystem drivers of aboveground communities like plant productivity and species diversity. Xue et al. (2018) also highlighted the spatial variation of soil microbial communities between different soil types as a result of external environmental mechanisms. Microbial diversity and abundance have been widely recognized as key ecological contributors toward ecosystem maintenance (Delgado-Baquerizo et al., 2015). It is, therefore, essential to better understand the relationship between environmental components and the distribution of soil microbes; a study that has very minimally been touched upon in the Middle East. The study conducted by Xue et al. (2018) suggested that the belowground microbial distribution is mainly associated with the soil chemical properties in Australia, while other factors such as climate, topography and temperature presented lesser influence. On the other hand, another study carried out in China indicated that the spatial distribution of microbial communities was largely influenced by the topography of different soil habitats and annual precipitation patterns (Tian et al., 2017). The results presented by Xue et al. (2018) also showed that agricultural and plantation fields reduced the overall diversity soil microbial communities and thus, contradicts those highlighted in

Lüneberg et al.'s study (2018). From this, it can be said that the factors and contributors influencing the distribution of soil microorganisms largely depends upon the region and local environmental components.

2.4 Soil Microbial Diversity

Biodiversity is often referred as the variability that exists among living organisms which integrates the ecological functions and biodiversity complexes within species as well as between species; with each living organism serving a fundamental function or biological role that supports the well-being of a given ecosystem (Maron et al., 2018). Soil biodiversity generally describes the variation of microorganisms including bacteria, fungi and archaea as well as other heterotrophic species that dwell beneath the soil surface. As a result, studies related to soil biodiversity is often neglected in ecological reports as little information is known regarding their specific role in ecosystem functions. Along with plantation and root systems, soil microorganisms interact with one another to form a network of biological activity (Mandal & Sathyaseelan, 2012). The biological fraction found in soil ecosystems equates to less than 0.5% of the total volume of soil. However, the biological activity carried out by these microorganisms are responsible for over 60% of global ecosystem services and functions including regulation of nutrient and carbon cycles as well as decomposition of organic matter (Laban et al., 2018). In most cases, micro-fauna such as bacteria and fungi are classified as detritivores due to their role in the decomposition process. Among its many functions, the role of soil biodiversity within the global cycle remains an integral part of ecology; research pertaining to soil microbiota is therefore, essential as it forms a basis to further understanding complex ecological relations.

Minor temperature changes can potentially alter the microbial diversity, which in turn, could directly influence soil integrity, plant community and geochemical

cycling (Mandal & Sathyaseelan, 2012; Wagg et al., 2014). Soil fungi are highly susceptible to environmental changes and are therefore, thought to be effective bio-indicators of the overall health of a given ecosystem (Laban et al., 2018). For instance, changes in global temperature and relative humidity will likely alter belowground fungal diversity and as a consequence, effect biological processes that may be detrimental to ecosystem functions and survival of aboveground species (Maron et al., 2018). Fungi matter contributes to a wide variety of ecosystem services and overall health of a given habitat; acting as the primary component in nutrient cycling, carbon sequestration and modification of soil chemical properties. The performance and productivity of an ecosystem is dependent on the complex interactions between microorganisms and plant matter deep within the soil (Xue et al., 2018). Microorganisms found within the soil aggregates are key components to the stability of soil food web as they perform critical functions like degrading toxic waste and acting as biological drivers for nutrient cycling whilst contributing to soil structure. Additionally, certain species of soil microbiota may also form symbiotic aggregates with plant roots and actively prevent pathogenicity of active plants by using their antagonistic nature to inhibit successful growth of harmful pathogens (Otsing et al., 2018).

The soil environment is characterized by a highly heterogeneous habitat of microbiota as varying soil components (organic matter, salinity and nutrients) and physical fractions (sand, clay and silt) provide multitudes of different microhabitats (Van Elsas et al., 2002). The extent of variation in soil nutrients and abiotic conditions may even vary in micrometre scale. It is hypothesized that a “stable” microhabitat is one that is inhabited by microorganisms that are best adapted to the niche (Garbeva et al., 2004). These microorganisms in turn, serve as the underlying biological drivers of

biochemical processes within the soil environment. Thus, it is evident that soil microbial processes take place at the scale of microhabitats. These micro-processes in turn, are receptive to environmental changes; whereby marginal shifts may result in a measurable effect in soil microbiome (Garbeva et al., 2004). The microbial biodiversity of a given area can be identified according to the number of different species and their abundance within the given habitat. In terms of molecular-ecology, it can be evaluated according to the number of different sequence types present in the genomic DNA extracted from a given soil environment (Garbeva et al., 2004). While an area inhabiting more species is generally regarded to be highly diverse, the evenness of species distribution should also be considered; an even distribution of microbial communities from one habitat is more diverse than compared to that of an unevenly distributed community from another habitat (Hedrick et al., 2000).

2.4.1 Soil Fungi and Ecosystem Functioning

Commonly known for its role in decomposition and nutrient cycling, fungi are successful soil inhabitants due to their plasticity and adaptability to adverse environmental conditions (Otsing et al., 2018). Conversion of organic matter into organic acids, biomass and carbon dioxide by fungi remains to be one of the most crucial processes in maintaining ecosystem functioning. All forms of soil may be defined according to its physical, biological and chemical properties, but the ability of a given soil system to thrive and adapt to unfavourable environmental changes driven by natural selection or anthropogenic influences is unique to the its properties (Treseder & Lennon, 2015). Many fungal species are assessed by their efficiency as a biosorbent for toxic metals like cadmium, mercury and lead. Though these pollutants may inhibit their growth, many fungal soil communities still remain to be an important biosorbent in the environment (Baldrian, 2003). Fungal diversity and activity in the soil is

regulated by a combination of biotic (agriculture, other microorganisms) and abiotic (soil pH, salinity, temperature) factors (López-Bucio et al., 2015; Rouphael et al., 2015).

In most cases, soil fungi are typically classified into three functional groups: (1) ecosystem regulators, (2) biological controllers and (3) organic matter decomposers (Frac et al., 2018). Ecosystem regulators are those responsible for maintaining soil health and quality by regulating physiological processes within the soil environment. Various mechanisms mediated by symbiotic soil fungi such as AMF that are shown in Figure 1. On the other hand, biological controllers regulate potential diseases and other pathogenic microorganisms. For example, Frac et al. (2018) reported an increase in plant growth in the presence of mycorrhizal fungi by improving nutrient uptake and protecting them against harmful pathogens. Fungal communities are strongly driven by the composition and diversity of crops and in turn, affect crop growth and reproductivity through mutualism or pathogenicity (Frac et al., 2018). Soil fungi also performs a pivotal role in nitrogen fixation, hormone production and soil stabilization (Treseder & Lennon, 2015).

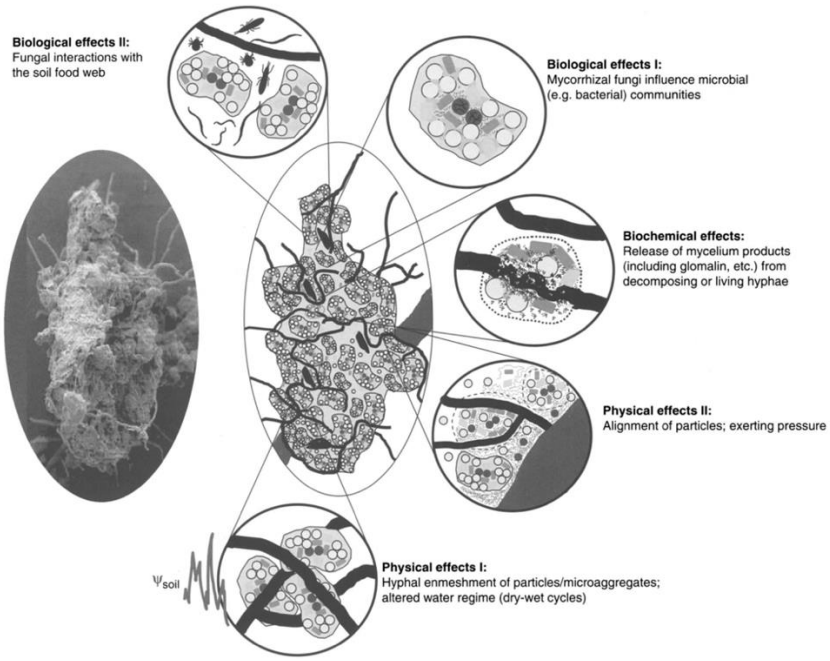

Figure 1. Overview of fungal microaggregates in soil and various physical, chemical and biological mechanisms mediated by fungal interactions (from Rillig & Mummey, 2006)

2.4.2 Fungal Phylogeny

The fungi kingdom has been delimitated into different clades according to biochemical, morphological and molecular-based studies; the most common phyla being *Basidiomycota* and *Ascomycota* (Schüβler et al., 2001). These fungal phyla have been studied extensively and thus, have relatively distinct lineage and phylogenetic classification. On the other hand, the phylum *Zygomycota* has been described as being polyphyletic, that is, a phylum that is derived from multiple evolutionary groups, and thus, may not be defined by a clear phylogenetic clade (Benny et al., 2001; O'Donnell et al., 2001; Schüβler et al., 2001). Based on Schüβler et al.'s study (2001), a comprehensive SSU rRNA analysis indicated a new fungal phylum, *Glomeromycota*, which represents monophyletic AMF. *Glomeromycota* phylum contains over 150 species (Walker & Vestberg, 1998) and shares common ancestral phylogeny with

Ascomycota and *Basidiomycota* phyla. A visual representation of the phylogenetic gaps between various fungal phyla is shown in Figure 2.

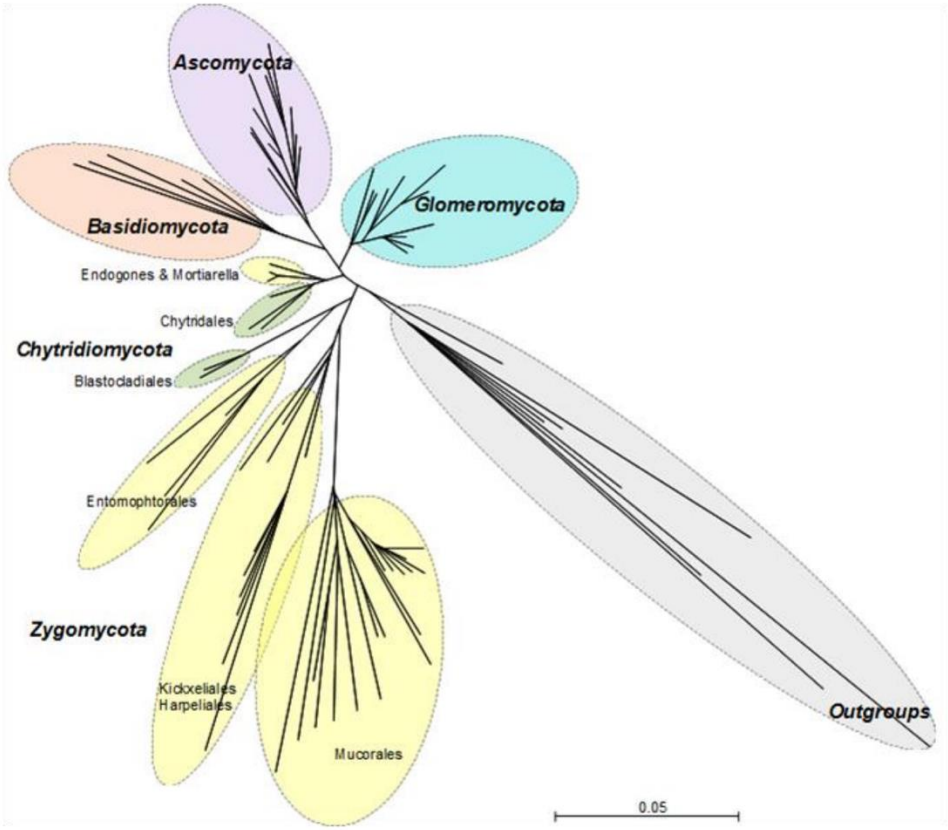

Figure 2. Phylogenetic tree of fungi based on SSU rRNA sequences. The fungal phylogeny indicates the relative position of *Glomeromycota* phylum among other fungal phyla. *Chytridiomycota* and *Zygomycota* are polyphyletic while *Basidiomycota*, *Ascomycota* and *Glomeromycota* form monophyletic clades (modified figure from Schüβler, Schwarzott, & Walker, 2001)

2.4.3 Arbuscular Mycorrhizal Fungi

Semi-arid and hyper-arid regions make up to almost 41% of the global ecosystems (Reynolds et al., 2007); most of which have been subjected to extreme desertification due to environmental stressors and anthropogenic impacts that has cultivated a decline in forests and forage species and accelerated changes in soil fungal

communities (Mahmoudi et al., 2019). While changes in plant communities are better studied (Mahmoudi et al., 2019), the effect of environmental changes on soil microbial communities and their role in arid habitats have yet to be better understood. Studies on soil microorganisms exhibiting direct interaction with plant communities such as arbuscular mycorrhizal fungi (AMF) may be of particular importance as a means of understanding soil and vegetation response factors towards environmental changes, particularly those resulting from climate changes (DeBellis et al., 2019).

AMF belong to the phylum *Glomeromycota* and forms an obligatory symbiosis with plants (Schüβler et al., 2001) and thus, plays a vital role in plant productivity. Schüβler et al. (2001) defined the *Glomeromycota* as a distinct entity based on fungal phylogeny and taxonomy. Based on Schüβler et al.'s (2001) taxonomic classification, AM fungi are considered to be monophyletic; that is, a group of fungal species that originated from a common ancestry. Figure 3 illustrates the phylogenetic tree of *Glomeromycota* phylum and various family groups within the AMF phylum. This information was based on a comprehensive SSU rRNA analysis which supersedes Smith's classification (1997) of *Glomeromycota* as being a new class within *Zygomycota* phylum.

According to Van Der Heijden et al. (2008), AM fungi enhances phosphorous acquisition and soil aggregation by improving soil structure and fertility. Additionally, symbiotic AMF may also promote plant growth and resilience by increasing its tolerance towards external biotic and abiotic stresses (Pozo & Azcón-Aguilar, 2007). AMF are especially crucial in fragile ecosystems as access to soil resources are limited and thus, are of particular importance in semi-arid and hyper-arid ecosystems (Zhao et al., 2017). In this regard, the diversity of AMF in arid environments may be used to establish symbiotic associations and consequently, preserve agricultural productivity

and ecosystem functions from desertification (little to no precipitation, high temperatures and dry seasons) (Mahmoudi et al., 2019; Zhao et al., 2017). However, the extent of mycorrhization depends on the plant species and AMF community in symbiosis. It was reported that a diverse AMF community can positively affect the diversity and productivity of plant ecosystems (Van Der Heijden et al., 2008). Moreover, plant associations with more diverse taxa of AMF improved its growth by reducing plant susceptibility to environmental stress and enhancing acquisition of soil resources in arid ecosystems (Mahmoudi et al., 2019).

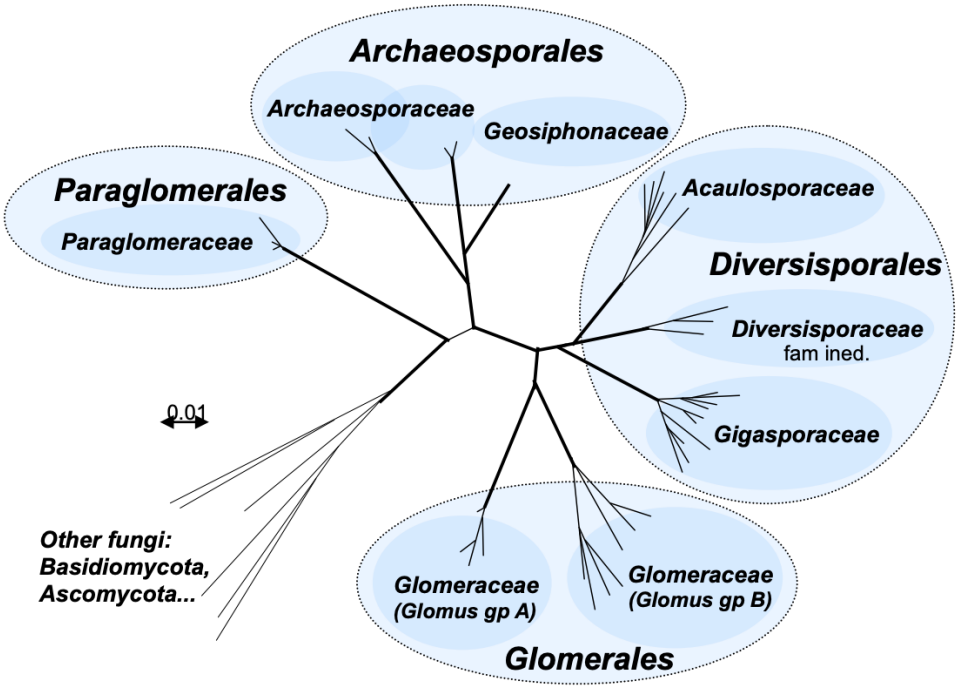

Figure 3. Phylogenetic tree of Glomeromycota phylum based on SSU rRNA sequences. The phylum represents four orders and eight families (modified figure from Schüβler et al., 2001)

The composition of soil AMF communities are highly influenced by the physical and chemical properties of the soil itself (as displayed in Figure 4, Jeffries et al., 2003). Soil disturbances influenced by agricultural management could positively

impact the diversity of AMF if agro-system management is associated with increasing soil organic matter and organic carbon (Oehl et al., 2010). Alternatively, a disturbance associated with decreasing organic matter will likely results in reduced diversity of AMF (Toljander et al., 2008). Previous studies have also shown that, rather than the type of agro-system management, the frequency of the established management has a bigger impact on AMF diversity (Mendoza et al., 2011). For example, the quantity of grazing species and intensity of grazing can produce distinct changes on the abundance and diversity of AMF communities. However, environmental changes such as increasing aridity caused by global climate change will generally reduce AMF diversity and abundance. This reduction in AMF diversity is most often associated with decreasing availability of soil carbon and nitrogen that comes with increasing aridity as primary production becomes increasingly restricted over time (Delgado-Baquerizo et al., 2016).

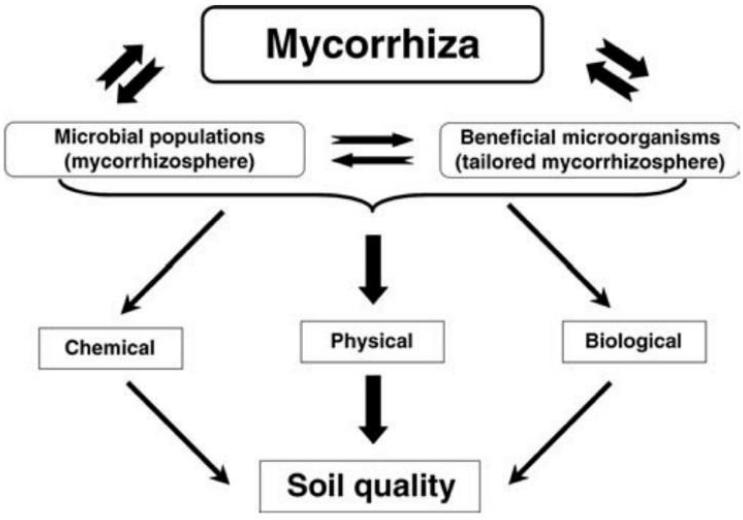

Figure 4. Interaction between arbuscular mycorrhizal fungi with natural and introduced soil microorganisms, which in turn affects the overall soil quality (from Jeffries et al., 2003)

2.5 Anthropogenic Influences on Soil Diversity in Qatar's Dryland

With increasing global population, the impact of anthropogenic disturbances on the natural state of a given soil habitat remains poorly understood. For instance, studies on the response of soil fungi and bacteria from climate change in Qatari drylands is essentially non-existent. However, Delgado-Baquerizo et al.'s study (2015) on global drylands suggests that with increasing aridity, soil fungal diversity is likely to reduce due to the negative influence of aridity on the nutrient availability and organic carbon content. Increasing aridity as a result of climate change stimulates a gradual shift in the composition of soil microbiota. It was found that highly-arid drylands shifted bacterial composition by increasing the relative abundance of Proteobacteria and decreasing Acidobacteria and Verrucomibria species (Delgado-Baquerizo et al., 2015). Changes in aridity and climatic conditions of a given soil ecosystem, such as those proposed in climate change models, could potentially reduce species diversity and richness and in turn, affect key ecosystem functions and nutrient cycles in drylands. In this regard, it can be assumed that climate change is a major driver of soil fungal diversity. Given the present emission rates of greenhouse gases and climate changes, climate-driven ecosystems can dramatically reduce processes like carbon sequestration, decomposition and nutrient cycling, thereby leading to a loss in soil biodiversity.

According to Neilson et al. (2017), reduced soil biodiversity in Atacama Desert drylands, Chile as a result of increased aridity has led to deteriorating soil aggregations and root associations between plantation structures and belowground microbial assemblages; a phenomena that could compromise the resilient nature of hyper-arid microbial communities as they lack the proper biochemical properties required to maintain its functional integrity. Anthropogenic activities such as combustion and industrialization that leads to a rise in soil temperatures and reduction in relative

humidity acts as contributing factors that significantly reduces soil fertility and microbial diversity. As a result, climate change driven variations in the temperature and aridity of a given soil habitat could pose a threat to the metabolic activity of soil microbes as it impedes the capacity of these microorganisms to function optimally, thereby threatening the productivity of the already fragile, arid lands. Neilson et al. (2017) reported that global warming and anthropogenic activities is expected to negatively impact the livelihoods of approximately 250 million individuals worldwide as well as expand the current surface coverage of arid land areas. Fungal activities in arid drylands is mostly characterized by both the chemical conditions of the land and its interactions with existing plantations and other macrofauna. Present soil microorganisms in desert regions such as those in Africa and the Middle East are functionally distinct from other biomes. Dryland regions of higher temperature or aridity exhibit fewer microbial species and are typically more susceptible to changes within its phylogenetic structure (Neilson et al., 2017).

The impact of anthropogenic activities on soil structure and soil microorganisms remains poorly understood. Human disturbances to natural ecosystems via land use, urbanization and unsustainable agricultural practices can negatively influence the structure of soil microorganisms. Land use is widely-considered as a determinant factor impacting the soil stability by altering soil chemical properties and nutrient availability, which could ultimately result in an increase or decrease of the soil fungal diversity. For instance, some studies have suggested that agricultural practices exhibit greater positive effects on the activity of microbial communities than compared to soil characteristics and precipitation (Xue et al., 2018). Current literature includes information on the distribution and diversity of soil microbiota in relation to changing

environmental parameters, however, little to no data is available regarding the influence of environmental elements on microbial diversity in the Middle East region.

Given Qatar's hyper-arid and hyper-saline environmental conditions that is highly susceptible to thermal fluctuations, fungal communities inhabiting Qatari soil will likely exhibit unique adaptations that may be of benefit for future biotechnological or molecular advances. Thus, delimitation of these key soil fungal species and defined environmental influencers can potentially serve as the first step towards understanding the role of fungi in biogeochemical cycles (i.e. carbon cycle), management of Qatari agricultural lands and biodegradation of soil pollutants.

Heterogeneity of land use in Qatar will often influence the microbial diversity which in turn, will affect the stability and physical structure of soil aggregates and biological interactions that shape these complex micro-environments (Majid et al., 2016). Moreover, the extent of fungal diversity present is adapted to the chemical and structural properties of the soil itself. The micro-environment of soil in Qatar (hyper-aridity, hyper-salinity and high thermal variability) favours certain forms of microbes that is highly adapted to Qatar's harsh environmental conditions. Identification of microbial diversity in Qatari soil will allow us to assess the function of these fungi present within the micropores of soils as well as their ecological interactions. The diversity of soil microbes is often associated with the chemical composition and availability of organic nutrients in the soil (Delgado-Baquerizo et al., 2015). Due to the oligotrophic nature of Qatari soil, we can assume that the presence of microorganisms within the soil structure functions as the central component that allows plants and Qatari flora to grow and flourish despite the harsh conditions of Qatar's natural environment.

2.6 Molecular Identification of Soil Fungi

Traditionally, the taxonomy of soil fungi has been determined according to its morphology, whereby families and genera were identified by assessing the mode of spore formation, while species were distinguished on the basis of spore size, colour, cellular structures and phenotypic characteristics of each spore formed (Datta & Kulkarni, 2012). In the case of AMF, the International Culture Collection of Vesicular AMF (INVAM) provides a comprehensive source of fungal classification, species morphology and taxonomy (http://invam.wvu.edu/the-fungi). However, accurate delimitation of fungal species may be challenging when spores are degraded, indicating dimorphic forms or when fungal spores are presented during its developmental phases. In this regard, molecular methods of fungal identification may be applied to overcome the limitations that comes with culture-based morphological methods of identification. Molecular identification of fungal species through DNA barcoding has become increasingly popular in recent years as studies pertaining fungal abundance and species richness from environmental samples have been made easier, while achieving more reliable results compared to those derived from culture-based approaches (Bellemain et al., 2010).

Application of DNA-based molecular tools is the most suitable method for studying fungal diversity within a community and among fungal species. Such techniques are continuously amended and improved to produce the best possible sequencing results. Nuclear-encoded DNA sequences are widely generated in the field of molecular phylogeny and taxonomy. The development of high-throughput sequencing (HTS) techniques has generated a significant change in methods of soil analysis and plant-associated fungal diversity. Standardized protocol for preparation of soil samples for high-throughput sequencing techniques (HTS) like Illumina, PacBio

and Ion Torrent has become widely available (Frac et al., 2018; Schöler et al., 2017). This cultivation-independent method of fungal identification generates files with millions of sequences within a shorter period of time; eliminating the need to cultivate and isolate fungal samples.

2.6.1 ITS as a DNA Barcode for Fungal Diversity

The internal transcribed spacer (ITS) region of nuclear DNA (nDNA) is commonly used as a barcode or DNA marker for the identification of single eukaryotic species and consortium of environmental DNA. The Smithsonian Conservation and Research Centre have considered ITS as the primary DNA marker for fungi after deliberation between 37 mycologists across the world (Bellemain et al., 2010). Over 100,000 ITS sequences have been generated using the standard Sanger sequencing and NGS; all of which are compiled in the International Nucleotide Sequence database (Nilsson et al., 2009), thus providing a large array of reference material for fungal identification. The ribosomal DNA (rDNA) region consists of three RNA-coding genes: the 18S small subunit (SSU), 28S large subunit (LSU) and the internal transcribed spacer (ITS) that is positioned between SSU and LSU. In terms of phylogenetic analyses, the SSU and LSU regions only show minor differences among closely-related species and thus, serves as a suitable DNA marker when determining distant relationships between species (Wu et al., 2007). On the other hand, in-depth analyses of the ITS region allows robust phylogenetic evaluation at the species level due to the high extent of polymorphism (Gamper et al., 2009).

Bellemain et al. (2010) assessed the extent of primer specificity to targeted sequences during PCR amplification and potential mismatches in certain taxonomic groups and consequently, suggested that the specificity of amplification of targeted sequences varies between ITS primer pairs. ITS3-ITS4 and ITS5-ITS2 showed to be

the most suitable pairs of primers to amplify the targeted fungal ITS region. Moreover, Bellemain et al. (2010) also concluded that primers ITS1 and ITS5 preferred amplification of basidiomycetes, while primers ITS2, ITS3 and ITS4 preferred amplification of ascomycetes. Though the entirety of ITS region has been verified as the universal barcode for fungi, there is no deliberation regarding the optimal ITS subregions (that is, ITS1 and ITS2) and primers used for high-throughput sequencing (HTS) technologies (Tedersoo & Lindahl, 2016). However, previous studies suggested that ITS1 region yields lower phylogenetic outcome than ITS2 region (Op De Beeck et al., 2014; Tedersoo et al., 2015). Figure 5 illustrates various targeted binding sites of the SSU-ITS-LSU region of rDNA using different primers. Combined with PCR biases, sequences recovered using ITS1 marker may yield lower fungal diversity (Tedersoo & Lindahl, 2016). Despite that, the use of ITS sequences may be used to evaluate fungal diversity in environmental samples, thereby generating a rough estimate across all eukaryotic groups. For this reason, Tedersoo & Lindahl (2016) proposed the use of the optimized primer ITS4ngsUni (21 bases), which accounts for almost all fungi mismatches and generates sequences with greater accuracy as it covers the entire ITS region (Kõljalg et al., 2013). In combination with ITS4ngsUni, the forward primer ITS9MUN (17 bases) (Egger, 1995) may be used to allow better taxonomic identification of fungal classes (Ihrmark et al., 2012).

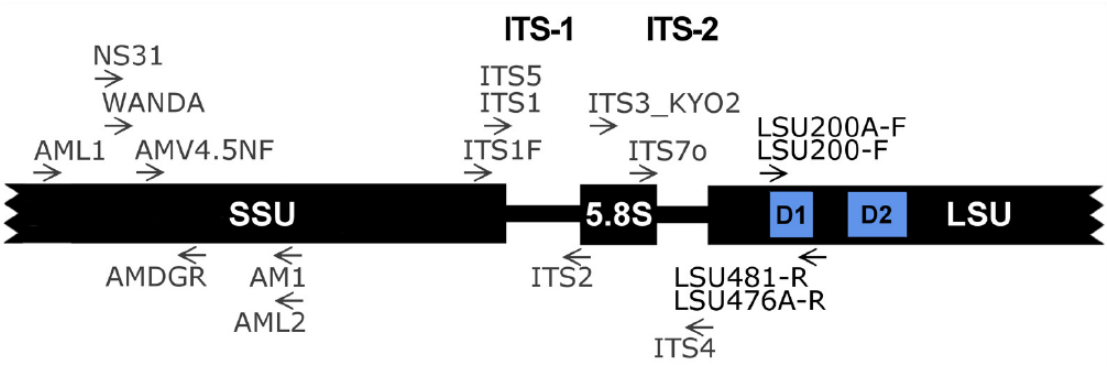

Figure 5. Small subunit (SSU), internal transcribed spacer (ITS) region and large subunit (LSU) region of rDNA with binding sites of various forward and reverse primer pairs as illustrated (from Asemaninejad et al., 2016)

2.6.2 High-Throughput Sequencing Platforms

Identification of fungi in natural ecosystems through pure culture and morphology has proven to be a difficult task due to the microscopic nature and cultivability of the microorganism (Vasar et al., 2017). More recent studies on fungal diversity has replaced morphological identification with DNA-based methods on the basis of fungal structures derived from environmental samples (Maarja Öpik & Davison, 2016). With the development of various HTS platforms, the substantial increase in the depth of sequencing (number of sequences per sample) has allowed in-depth analysis of the abundance and variation between fungal communities including AMF. Platforms like Illumina sequencing have gained increasing recognition in recent fungal diversity research due to its low error rate and enhanced sequencing depth per sample (Shokralla et al., 2012; Tedersoo et al., 2015; Vasar et al., 2017). For example, in one study, Illumina sequencing detected low AMF diversity in comparison to the sequencing depth of 454 sequencing system; accounting for the impact of extreme environmental conditions (Vasar et al., 2017). Alternatively, another study focusing on AMF communities in grass roots indicated high sequencing recovery with Illumina, while covering a wide range of phylogenetic clades (Johansen et al., 2016). Moreover,

results from dryland ecosystems in Northern China (Wang et al., 2016) also showed that Illumina sequencing techniques can recover large quantities of AMF sequences.

The ongoing challenge in many diversity research studies is pairing the targeted genetic markers with the appropriate primers while ensuring its effectivity in sequencing technologies. Among the different NGS technologies, Illumina MiSeq is considered to be the most effective NGS method globally at a relatively low cost. This method, however, requires short amplicon regions of 300 base pairs; Illumina sequencing of longer regions will therefore, reduce the overall accuracy (Asemaninejad et al., 2016). The limitations that comes with NGS technologies renders it unsuitable for certain biological analysis including determination of more complex genomic regions and detection of methylation (Rhoads & Au, 2015). Although second-generation sequencing (SGS) techniques like Illumina has become the most common approach for microbial analysis, the relatively short sequence length (100-500 bp) generated by such platforms may be a major deterrent as the taxonomic outcome of shorter DNA barcodes are less accurate compared to that of the original DNA barcode (Kõljalg et al., 2013; Schlaeppi et al., 2016).

On the other hand, unlike SGS platforms, third-generation sequencing platforms like Pacific Bioscience (PacBio) generate sequences of genomic barcodes of up to 60 kbp (Tedersoo et al., 2018). Although in comparison with SGS tools, third-generation PacBio technology produces higher initial error rate and relatively low throughput sequences. However, the ability of PacBio systems to provide longer sequenced data has rendered it to be a useful approach in many taxonomic studies (Rhoads & Au, 2015). Tedersoo et al. (2018) reported greater taxonomic success of fungi and other eukaryotes when sequencing full ITS regions compared to sequences of ITS1 and ITS2 subregions – taxonomic identification using PacBio system (sequence of full ITS

region) showed 33% greater success according to the genus level and 9% according to the phyla. Therefore, despite the poor quality of sequence, the PacBio platform may be a viable alternative for phylogenetic identification that require longer amplicons.

2.7 Aims of the Study

The aim of this research study was to identify soil chemical drivers of AMF communities. Various locations around Qatar with differences in topography, land type and vegetation coverage were studied to evaluate AMF species diversity and determine dominant indicator species. Fungal species was identified by Illumina MiSeq sequencing to evaluate the diversity and abundance of soil fungi and consequently, link the chemical contributors including land type with the fungal community in Qatar's soil. The final aim is to evaluate differences in soil fungal diversity and physicochemical characteristics between study sites and identify potential dissimilarities within each site. This concept is particularly important in climate-driven ecosystems as potential changes in aridity due to climate change may influence the ability of dryland soil microbiomes to sustain geochemical cycles and ecosystem functions (Delgado-Baquerizo et al., 2015). Climate-driven changes in soil chemical properties and fungal diversity may also compromise the overall health of vegetation areas in Qatar. This study is therefore, essential to fully understand the influence of soil chemical parameters and heterogeneity of Qatar's land on fungal communities and in turn, its influence on plant fertility and stability.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area, sample collection and processing

In order to study the association between fungal diversity in Qatar's soil environment and soil chemical characteristics, the sampling sites were selected from 19 locations of varying topography, vegetation coverage and soil conditions. Much of Qatar's land is dominated by large desert areas with a high arid climate and minimal annual rainfall that often occurs sporadically. Qatar's topography consists of mainly flat land surfaces and undulating sand formations that mostly occurs in the south-east region. The soils are abundant in calcareous rocks, sands and gravel. Qatar is a predominantly low-lying country with small mountainous regions (i.e. jebel) in the south of Qatar that reach just over 100 m in altitude. Additionally, Qatar's land also comprises of several natural depressions (i.e. rawdha) with soils that are often deeper and richer (Norton et al., 2009). The locations presented different land types including saltmarsh, mangrove, rawdha, sabkha and vegetation regions in the central and coastal regions of Qatar. Some sampled locations were within close proximity to commercial districts and urban areas, while other sampled soils were collected from undisturbed areas; those are unaffected by human disturbance. Each location sampled in this study was categorized according to the type of land based on ArcGIS MDAUS BaseVue 2013 metadata (https://arcg.is/0j81yG). Coordinates of the study locations are presented in Table 1.

Soil samples were collected from 19 study sites (approximately 50 m x 50 m). From each site, 20 soil samples were collected from vegetated spots, 5 cm in diameter and 5 cm deep in the soil, following the protocol for a global project on soil decomposers and mycorrhizal fungi (Tedersoo personal communication). From each site, composite samples from the 20 subsamples were prepared by combining

approximately 1-1.5 g of each subsample per site. The composite soil samples (approximately 10 g) were left overnight to air-dry and stored in zip-lock plastic bags with silica gels to prevent moisture. In addition, from five of the 19 sites, the individual soil core samples were kept to analyze potential variations in chemical and fungal composition within each site. Thus, there was 119 soil samples for chemical and genetic analysis (19 composite samples, and 20 (total 100) samples from five sites). As plants have the capacity to form a mycobiota of beneficial fungi (Zarei et al., 2010), sampling of soil samples close to areas of vegetation or plant abundance should give a useful overview on the fungal community in each study site. Soil samples prepared for molecular analysis were neither sieved nor ground in a ball miller in order to avoid contamination and potential loss of genetic material. However, soil samples were manually crushed by hand to disrupt any soil colloids for downstream molecular analysis (Tedersoo personal communication).

For chemical analysis, 29 soil samples comprising of 19 composite samples, two duplicates and three subsamples were dried inside an oven at 60-62^{o}C for 48 hours to prevent further decomposition of organic material and enhance the extractability of micronutrients and heavy metals (Erich & Hoskins, 2011). The dried soil is then grinded into fine dust in a rotary ball mill (Retsch PM400 Planetary Ball Mill) at a speed of 250 rpm for 40 minutes and consequently, passed through a standard sieve of 2 mm mesh in preparation for downstream chemical analysis.

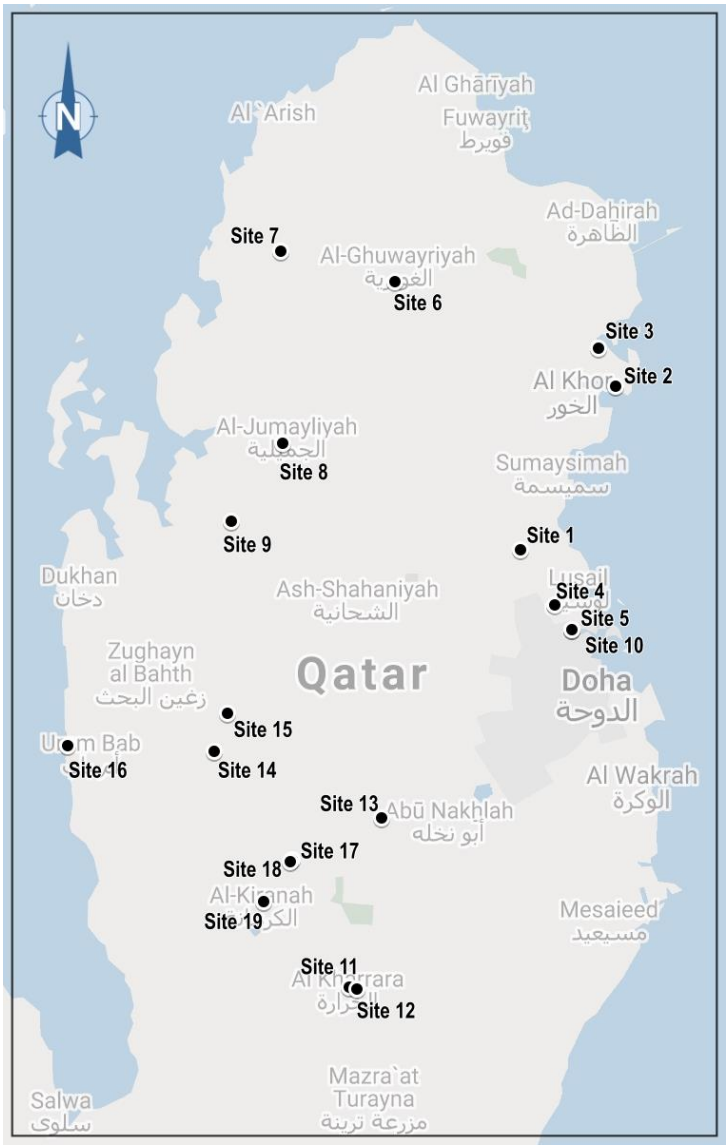

Figure 6. Locations of 19 study sites across Qatar. Due to the close spacing of some study sites, the symbols overlap and are therefore, indistinguishable

Table 1. Descriptions of each sampling site including dominant plant taxa

Site no.*	Site name	Latitude	Longitude	Dominant plant at site	Site description
1	AlSakhama	25.476034	51.421473	*Prosopis juliflora*	Sandy area with intermittent rocks and patches of vegetation
2	AlKhor	25.687524	51.557793	*Avicennia marina*	Mangrove area with extensive halophytic plants in clay/loam soil
3	AlThakira	25.737380	51.534113	*Halopeplis perfoliata*	Saltmarsh with partial vegetation coverage
4	Lusail	25.404546	51.470202	*Acacia sp.*	Wet muddy soil with patches of vegetation as a result of rainfall
5	QU field 1	25.371027	51.496573	*Stipagrostis plumosa*	Extensive grassland area that is however, disturbed as it is in close proximity to the city
6	AlGhuwayriyah	25.825498	51.239716	*Prosopis juliflora*	Sandy area scattered with bushes and trees attributed to rainfall
7	AlNu'man	25.864567	51.075506	*Senna italica*	Rawdha area dominated with trees and shrubs
8	AlJumayliyah	25.615461	51.078010	*Astragalus sp.*	Slightly moist soil with scattered areas of vegetation and little rocks
9	Khawzan	25.512379	51.002956	*Stipa sp.*	Moist and well packed soil with small plants and patches of grass
10	QU field 2	25.372192	51.495583	*Zygophyllum qatarense*	Sabkha area dominated by rocky soil and trees
11	AlKharrara 1	24.903225	51.173316	*Rhanterium epapposum*	Rawdha area filled with trees and scattered shrubs. The area is inhabited by grazing animals
12	AlKharrara 2	24.902584	51.185224	*Prosopis juliflora*	Rawdha area filled with trees and scattered shrubs

Site no.*	Site name	Latitude	Longitude	Dominant plant at site	Site description
13	AlRayyan	25.124299	51.221968	*Prosopis juliflora*	Largely populated by large bushes, flowering plants and grass patches
14	AlKharsaah 1	25.212063	50.979881	*Lycium sp.*	Grassland with several trees. Likely a disturbed area due to its close proximity with the roadside
15	AlKharsaah 2	25.261641	50.998017	*Ziziphus sp.*	Rock outcrops with patches of grass
16	Dukhan beach	25.218835	50.767926	*Halopeplis perfoliata*	Coastal area dominated by halophytes and fine sandy soil
17	North of Qatar 1	25.06971	51.09184	No dominant plant	Desert area with plain flat soil and minimal vegetation
18	North of Qatar 2	25.06915	51.08974	No dominant plant	Desert area with little to no vegetation
19	AlKiranah	25.01562	51.04944	No dominant plant	Area with dunes and deep sand

*Each site number corresponds to the pinned locations shown in Figure 6

3.2 Soil Chemical Analyses

Collected soil samples were analysed for eight key chemical parameters (see Table 3 for a summary of the analysed parameters and methods used for each analysis). These measured components were used to determine the chemical nature of soils collected around Qatar and identify potential differences between the chemical components of soil composites and sub-samples.

3.2.1 Determination of Soil pH and Conductivity

The portion of processed soil designated for chemical characterisation were analysed for the parameters: pH, electrical conductivity (EC), salinity and total dissolved solids (TDS) using a probe meter. To measure the pH, a 1:1 ratio of soil to water mixture was prepared (5 g of soil and 5 ml of distilled water) and the pH of the slurry was measured using a pH probe meter (Mettler Toledo AG). The pH meter was calibrated using 7.0 and 4.0 buffer solution that was measured at 18.0^{o}C to adjust the accuracy of readings with temperature and sensitivity on the instrument. The pH probe was rinsed with distilled water between each measurement and kept in saturated KCl solution when not in use. Excess water was carefully dried with a tissue to ensure maximum quality in the readings between pH measurements.

The same prepared mixture was used to determine electrical conductivity, TDS and salinity of each soil sample by adding an extra 20 ml of distilled water to produce a 1:5 ratio of soil to water and consequently, kept in an orbital shaker at high speed for approximately 1 hour. Since the electrical conductivity is determined by the concentration of ionized constituents in solution, the soil suspension was left standing for several hours to allow complete separation of the liquid and solid phase (that is, the water and settled soil). The conductivity, TDS and salinity were measured by placing the EC electrode (EC300 YSI EcoSense conductivity meter) into the supernatant

(where the dissolved ions are present), while ensuring that the soil suspension is not disturbed. The EC meter was calibrated using 0.01M of KCl solution measured at 25^{o}C, which produced a conductivity of approximately 1.413 dS/m. The conductivity electrode was rinsed thoroughly with distilled water between measurements. Excess water was carefully dried with a tissue to ensure maximum quality in the readings after each TDS, EC and salinity measurement.

3.2.2 Determination of Total Carbon and Total Nitrogen by Combustion

The total carbon and total nitrogen in twenty-nine soil samples (both composite and sub-samples) were determined by combustion in a CHN elemental analyser (Skalar) following the approach mentioned by (Jing et al., 2015). Forty milligrams of glycine was used as a known standard control and calibration standards with known concentrations. A calibration curve was generated of the resulting absorbance values over the weight of the standards and subsequently, total carbon (TC) and total nitrogen (TN) were calculated accordingly. Recovery of the standards for TC and TN were 96.74% and 95.74% respectively and thus, falls within 15% of the acceptable range (as specified by EPA), to ensure the accuracy and reliability of the data achieved.

3.2.3 Concentration of Soil Nutrient by UV-Visible Spectrophotometry

3.2.3.1 Potassium chloride (KCl) Extraction of Nitrate and Nitrite

Approximately 2 g of dry soil matter (accurately weighed to .01 g) and 20 ml of KCl (2 M) were mixed in a 50 ml tube and vigorously agitated using a mechanical shaker for 24 hours to ensure complete extraction of NO_3^- and NO_2^- components. The resulting supernatant and soil suspension were separated by centrifuging at a speed of 3500xg for 10 minutes.

3.2.3.2 Preparation of Reagents and Standard Solutions

Nitrite ions react with sulfanilamide solution to form a diazo compound under acidic conditions. When coupled with N-1-napthylenediamine dihydrochloride (NEDD), the solution produces a reddish-purple azo dye. The intensity of the colour is directly proportional to the concentration of nitrite-nitrogen present. Absorbance of the azo dye was determined by UV-Vis spectrophotometry at a wavelength of 543 nm. A 0.5 g of sulfanilamide was dissolved in 5 ml concentrated hydrochloric acid and diluted to 50 ml of total volume. Similarly, 0.1 g of NEDD was dissolved in distilled water to 50 ml of total volume. $NaNO_2$ standard nitrite (for the calibration of NO_2-N) and $NaNO_3$ standard nitrate (for the calibration of NO_3-N) concentrations were prepared from 0 to 250 ppb and its absorbance values determined at 543 nm by spectrophotometry. The standard calibration curve was generated using the derived absorbance values for NO_2-N and NO_3-N respectively.

3.2.3.3 Determination of Soil Nitrate

The extracted soil suspension was centrifuged at 3500xg for 10 minutes prior to spectrophotometric quantification. 10 ml of the liquid fraction was transferred and added to 0.20 ml of both reagent solutions previously prepared (that is, sulfanilamide and NEDD solution). The mixture was left standing for a minimum of 10 minutes and maximum of 2 hours to allow complete reaction of the reagents and soil solution. Extinction or intensity of the mixture was then measured in a cuvette placed in a UV-Vis spectrophotometer (Thermo Scientific Evolution 201) at 543 nm. Calibration of the spectrophotometer was attained using a blank and standardization was achieved using previously prepared $NaNO_3$ standard solutions.

3.2.3.4 Determination of Soil Nitrite by copperized-cadmium Reduction

3.2.3.4.1 Preparation of Reduction Column

The method of soil nitrate analysis was described in (Wood et al., 1967) calorimetric method of nitrate determination. Nitrate concentration in soil solution is determined following its reduction to nitrite by percolating the extracted soil solution through a copperized-cadmium packed column. Approximately 40 g of cadmium filings were flushed with concentrated HCl and consequently, rinsed with distilled water. The cadmium filings were treated with copper sulphate solution and re-rinsed with distilled water to prevent cadmium exposure to air. Copperized-cadmium was slowly introduced into the column reservoir by inverting the cadmium cylinder. The column was also continuously tapped during the transfer process to settle the cadmium filings and consequently, left to stand for 24 hours minimum before use. Setup of the copperized-cadmium column apparatus is shown in Figure 7.

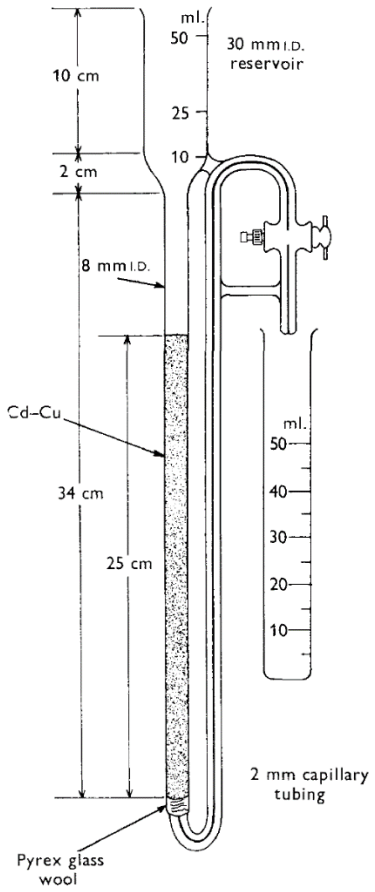

Figure 7. Apparatus setup of copperized cadmium column used to reduce nitrate to nitrite present in each soil suspension and nitrate standard solutions. 10 cm length between the place of discharge and bottom of the reservoir was necessary to maintain constant flow of sample solution so as not to introduce air into the column (from Wood et al., 1967)

3.2.3.4.2 Determination of Nitrite by Column Reduction

Prior to sample reduction, the cadmium column was flushed twice with 10 ml diluted ammonium chloride (NH_4Cl). The flow rate was adjusted to obtain approximately 100 ml of solution in 8 to 12 mins. 1 ml of concentrated NH_4Cl was added to 50 ml extracted soil solution and mixed vigorously. 10 ml of the resulting NH_4Cl-solution mix was used to flush the column twice before percolating the remaining sample through the column. 10 ml of reduced solution was collected for downstream nitrate quantification. Similar to (Benschneider & Robinson, 1952)

spectrophotometric approach of nitrite determination, 0.20 ml of both reagents was mixed with 10 ml of reduced sample and left standing for 2 hours to ensure complete reaction. Absorbance of the azo dye solution was determined by UV-Vis spectrophotometry (Thermo Scientific Evolution 201) at 543 nm. Calibration of the instrument was attained using a blank cuvette of distilled water and standardization was achieved using reduced $NaNO_3$ standard solutions.

3.2.4 Concentration of Trace Metals in Soil by ICP-OES

Preparation of soil samples for ICP-OES analysis was carried out by weighing 0.25 g of soil into a PTFE Teflon block digestion tubes enclosed with disposable reflux caps. Samples were digested with 9.0 ml of concentrated nitric acid (69% w/w) and 3.0 ml of concentrated hydrofluoric acid placed in a 54-well digestion hot block (Thomas Scientific, Environmental Express) at 135^{o}C for approximately 1 hour. The open hot-plate digestion method used in this study was presented by (Tighe et al., 2004) with minor modifications. Following acid digestion, evaporation was initiated by removing the reflux caps and increasing the temperature to 155^{o}C for an additional hour. Following near complete evaporation, 3.0 ml of nitric acid was added to the remaining residue and diluted to a total volume of 50 ml. Samples were heated until boiling point and consequently, transferred in a 100 ml volumetric flask and diluted with Milli-Q water ready for ICP-OES analysis.

The ICP-OES instrument used was a Perkin Elmer Optima 7300DV System fitted with an S10 autosampler. Samples were analysed for the trace elements: magnesium, potassium, phosphorous, calcium, cadmium and lead. Obtained data was processed using WinLab32 software. To ensure reliability of the data attained, internal standards were included during ICP-OES analysis to avoid potential interferences (Masson et al., 2010). Variable responses of each analytical element may reduce the

overall recovery; internal standards based on the ionization potential of known trace elements are therefore, incorporated during analysis to avoid such recovery error. Additionally, the accuracy of analytical method was verified by analysis of soil certified reference materials (CRM): PACS3-1 and PACS3-2. Recovery of the CRM analysed fell within the range 90-100%; the following recoveries of analysed trace elements were therefore, within acceptable range (±20%). Two soil samples were prepared in triplicates as well as two blank samples to ensure quality control (e.g. precision) during digestion and evaluate potential contamination during sample preparation.

Table 2. Summary of the methods used for analysing the chemical parameters

Chemical Parameter	Method	Reference	Instrument
pH (1:1; soil : water)	Potentiometry	(Patriquin et al., 1993)	Digital pH meter (FiveEasy Mettler Toledo)
EC (1:5; soil : water)	Conductometry	(Pinto et al., 2010)	Digital conductivity meter (YSI EcoSense model EC300)
TDS (1:5; soil : water)	Conductometry	(Tandon, 2005)	Digital TDS meter (YSI EcoSense model EC300)
Total carbon	Dry combustion	(McGeehan & Naylor, 1988)	CHN elemental analyser (Skalar Primacs)
Total nitrogen	Dry combustion	(McGeehan & Naylor, 1988)	CHN elemental analyser (Skalar Primacs)
Nitrate	Nitrate reduction followed by spectrometry	(Wood et al., 1967)	Copperized-cadmium reduction column followed by UV-Visible spectrophotometer (Thermo-Scientific Evolution 220)
Nitrite	Spectrometry	(Kelly & Love, 2007)	UV-Visible spectrophotometer (Thermo-Scientific Evolution 220)
Trace metals	Acid digestion followed by ICP spectrometry	(Tighe et al., 2004)	ICP-OES (Perkin Elmer Optima 7300DV System)

3.3 Molecular Analysis of Soil Fungi

3.3.1 DNA Extraction

Environmental DNA was extracted from 0.25 g of homogenized soil sample using DNeasy PowerSoil Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Consequently, the total genomic DNA was quantified and tested for extraction quality using 1% agarose gel electrophoresis. According to the bands shown on the gel, high quality DNA extracts of both composites and subsamples were used as templates in PCR amplification.

3.3.2 PCR, library preparation and sequencing

For identifying the total fungal and more specifically arbuscular mycorrhizal fungal species, forward and reverse primer pairs WANDA-AML2 respectively (Dumbrell et al., 2011; Lee et al., 2008) were used to target sequences in the SSU region of the rRNA gene (Table 2). The universal eukaryotic primer, WANDA, is located 23 bp downstream from NS31 and thus, brings the targeted region (i.e. AMF SSU rRNA) closer to the start of each amplicon (Dumbrell et al., 2011), while the Glomeromycota-specific primer, AML2, amplifies SSU rRNA sequences of all AMF phylogenetic classes, excluding *Archaeospora trapeii* (Lee et al., 2008). Each primer was tagged with 12 base multiplex identifier (MID) tag as described in Tedersoo et al. (2014). MID tags were modified versions of those recommended by Roche (Basel, Switzerland) and consist of 30 to 70% adenosine and thymidine to optimize adaptor ligation. All primers were ordered desalted from Microsynth (Balgach, Switzerland). Detailed information on the library codes and primer sequences can be found in Appendix F.

Table 3. MID tagged primers used to target the SSU region of the rRNA gene for high-throughput identification of fungi

Primer pairs	Sequences 5'-3'	Amplicon size	Target	Reference
WANDA	CAGCCGCGGTAATTCCAGCT	~550bp	AMF SSU rRNA	(Dumbrell et al., 2011)
AML2	GAACCCAAACACTTTGGTTTCC			(Lee et al., 2008)

Polymerase chain reaction (PCR) was carried out in a total reaction volume of 25 µl using 1 µl of template DNA, 0.5 µl of forward and reverse primer (20 µM), 18 µl of nuclease-free water and 5 µl of 5X Hot FIREPol Blend Master Mix (Solis Biodyne, Tartu, Estonia). The Master Mix blend consists of DNA polymerase, 0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, and 200 µM of each dNTPs. Optimal PCR conditions used for amplifying fungal communities were as follows: 15 min at 95ºC, followed by 35 cycles of 30s at 95ºC, 30s at 55ºC, 1 min at 72ºC and a final extension temperature of 72ºC for 10 mins. PCR was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The quantity of each PCR product was estimated by running 5 µl of amplicon DNA on 1.5% agarose gel electrophoresis for 30 minutes. PCR products that indicated no visible bands or weak bands on the gel were re-amplified with increasing number of cycles from the initial number of cycles (38-40 cycles for fungal communities), while PCR conditions were kept the same for each set of forward and reverse primers.

PCR products were pooled into two libraries per primer pair at approximately equimolar ratios as determined by the strength of the gel band and consequently, purified using FavorPrep gel/PCR purification kit (Favorgen Biotech Corp, Ping Tung, Taiwan) following the manufacturer's protocol. Each library was linked with Illumina

MiSeq adapters using the TruSeq DNA PCR-free HT Library Prep kit (Illumina Inc., San Diego, CA, USA) and processed by Illumina MiSeq 2X300 sequencing platform at the Estonian Genome Centre (Tartu, Estonia). Negative (nuclease-free water in place of template DNA in PCR mixture) and positive controls (DNA extracted from *Plantago ovata* roots) were used throughout the experiment including sequencing runs.

3.3.3 Data Processing and Bioinformatics

Illumina paired-end raw reads that were generated as fastq libraries, with some of the reads having overlapped ends, were processed using a series of bioinformatics steps (Vasar et al., 2017). Sequences were cleaned by selecting reverse and forward reads with an average quality of at least 25, however, both paired-end reads could have one mismatch in forward and reverse primer sequence. Quality filtered paired-end reads were combined with FLASH (v1.2.10, Magoč et al., 2011) using the default thresholds (that is, overlap between 10 bp-300 bp and a minimum overlap identity of 75%). Sequences were pre-clustered with the default identity percentage of 98% using VSEARCH (v2.14.1, Rognes et al., 2016) to reduce the processing time. Cluster information was stored for allowing mapping clusters back to individual reads. Putative chimeric sequences were removed using VSEARCH reference database (MaarjAM database (status February 2020, Öpik et al., 2010) and *de novo* chimera filtering algorithms. AM fungal sequences of the SSU region were matched for virtual taxa (VT; phylogenetically defined taxonomic units) using a BLAST+ search against the open access MaarjAM database (http://maarjam.botany.ut.ee), with 97% identity and 95% alignment thresholds. All VTs represented by a single sequence (singletons) were removed. The AM fungal sequences were matched to identify the virtual taxa using BLAST+ against MaarjAM database for a second time with 85% identity and 95% alignment thresholds.

3.4 Statistical Analyses

In terms of data analysis, the Shapiro-Wilk (S-W) test was used to examine the normal distribution of soil variables. Variables that did not pass the normality test at 0.05 significance level were normalized by logarithmic transformation prior to ANOVA. One-way ANOVA and Tukey variance test was then carried out to evaluate the significance between means at 95% confidence interval for each chemical variable. ANOVA analyses was conducted using SPSS 19.0 software (SPSS Inc., USA). The chemical variables and concentration of trace metals were assessed by Pearson's linear correlation matrix and subsequently, Principal Component Analysis (PCA). PCA was used to determine the extent of variability between the chemical components in different sites. PCA was also used to evaluate different combinations of chemical variables measured that can explain a common pattern of variation among the sites sampled in this study.

Shannon and Simpson alpha-biodiversity analyses were computed to determine the species diversity and richness of identified AMF genera. Alpha-diversity indices of AMF VTs were also determined according to the study sites to determine potential trends. The frequency of AMF occurrence at each location was determined according to the VTs of each genus identified. Agglomerative hierarchical clustering (AHC) was used to identify AMF clusters and analyse potential differences in AMF communities between the composite soil samples. An indicator species analysis was carried out to determine the AMF indicator index value for each study site as per the method of Dufrêne & Legendre (1997).

Using the variables obtained from initial analysis, a distance-based redundancy analysis (RDA) was carried out as per the method described in Zhao et al. (2017) to explore potential relationships between the composition of AMF community

assemblages, soil chemical characteristics and land-type (i.e. mangrove, saltmarsh, rawdha, barren land/sabkha and vegetation/shrub patches). Linear regression was conducted to evaluate the relationship between soil characteristics and AMF abundance. RDA and regression analyses were computed using XLSTAT statistical software (Addinsoft Inc., New York, USA).

CHAPTER 4: RESULTS

4.1 Soil Chemical Properties

4.1.1 TC and TN Concentrations by CHN Analyser

Using the area values determined, the absorbance of six glycine ($C_2H_5NO_2$) standards indicated that the absorbance of C (Table 4) and N (Table 6) is directly proportional to its weight. Accordingly, the absorbance values of 19 composite soil samples were also measured and consequently, used to extrapolate the weight of carbon and nitrogen content and consequently, calculate soil %TC and %TN present in each study site using the line equation derived from the standard calibration graphs. The calibration standards for the carbon and nitrogen content in glycine are shown in Figures 8 and 9 respectively. To ensure reliability of the values, the recovery between theoretical TC (Table 5) and TN (Table 7) in glycine and calculated values of the unknown samples was determined which fell within 15% of EPA criteria. A summary of the TC concentrations can be found in Table 11. TN concentrations were rendered as negligible since the TN values were well below the limits of detection.

Table 4. Absorbance area of standard carbon as determined according to known carbon content in glycine, $C_2H_5NO_2$

Standard	Wt. Glycine (mg)	Wt. C (mg)	Area
1	16.3	5.21111	1042955.1
2	80.1	25.60797	4552466.4
3	154.6	49.42562	8774903.555
4	228.3	72.98751	13101895.12
5	295.3	94.40741	16669673.88
6	367.5	117.48975	21137782.54

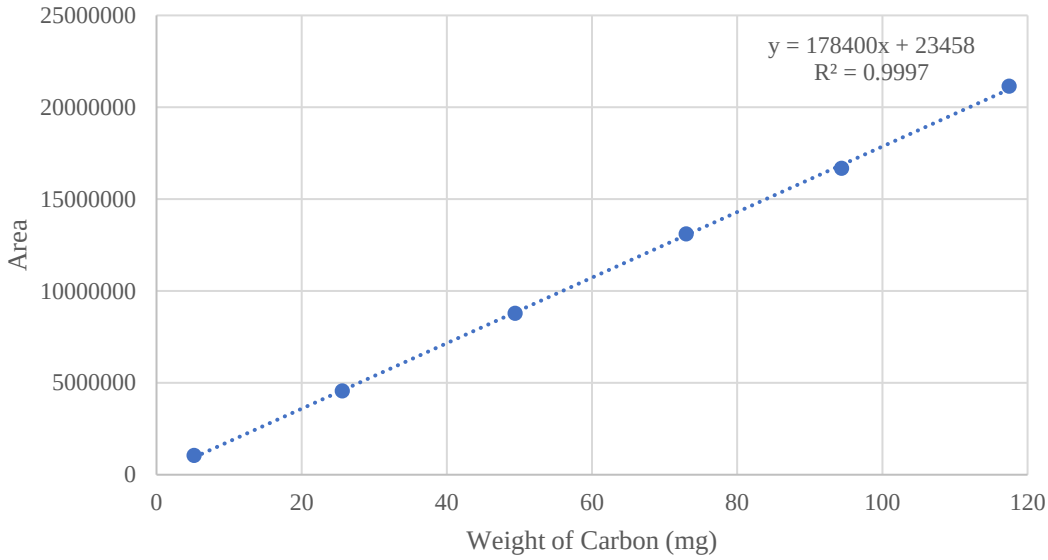

Figure 8. Standard calibration curve of carbon in glycine compound

Table 5. The recovery of TC measured in the standard glycine compound

Standard compound	Glycine
Wt. sample, mg	40
Area	2230548.24
Wt. C, mg	12.3715821
%C (as measured)	30.93
Theoretical Glycine	31.97
%Recovery	96.74
EPA criteria	within 15% of value

Table 6. Absorbance area of standard nitrogen as determined according to known nitrogen content in glycine, $C_2H_5NO_2$

Standard	Wt. Glycine (mg)	Wt. N (mg)	Area
1	16.3	3.03995	71689.79
2	80.1	14.93865	249694.05
3	154.6	28.8329	469050.735
4	228.3	42.57795	688586.065
5	295.3	55.07345	886725.86
6	367.5	68.53875	1104885.49

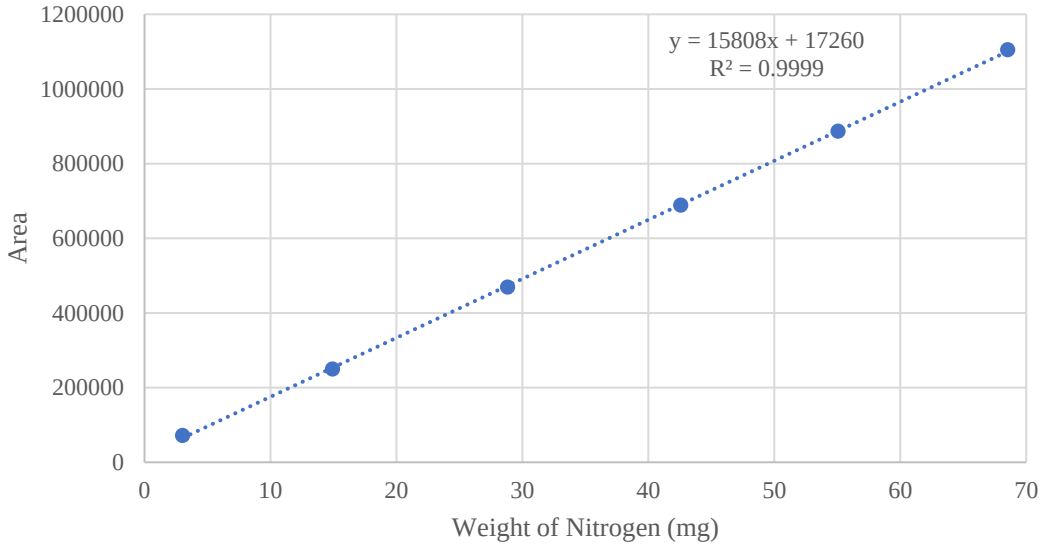

Figure 9. Standard calibration curve of nitrogen in glycine compound

Table 7. The recovery of TN measured in the standard glycine compound

Standard compound	Glycine
Wt. sample, mg	40
Area	130169.4
Wt. N, mg	7.1425
%N (as measured)	17.86
Theoretical Glycine	18.65
%Recovery	95.74
EPA criteria	within 15% of value

4.1.2 NO_2^- and NO_3^- Concentrations by UV-Vis Spectrophotometry

Spectrophotometric measurements of NO_2^- and NO_3^- was conducted by UV-Vis spectrophotometry at 543 nm. The concentration of nitrate and nitrite varied among different land types. The results achieved indicated significant fluctuations in the concentration of soil nitrate while the concentration of soil nitrite remained relatively constant among all study sites. Although there were no significant differences in the concentration of soil nitrite across all sampled sites, the highest quantity of nitrate was evidently found in the soil collected from QU fields. It was initially hypothesized that regions of high nutrient content are directly correlated with increasing diversity and abundance of AMF communities. Results presented in this study supported the initial presumptions made. Despite the results achieved, it should also be noted that the availability of nutrients in soil are highly heterogeneous in space and time.

The absorbance values of NO_2^- (Table 8) and NO_3^- (Table 9) standards were determined and a calibration curve plotted of known nitrate and nitrite concentrations against its average absorbance. The data obtained for the known standards indicated that the absorbance of NO_2-N (Figure 10) and NO_3-N (Figure 11) is directly proportional to its concentration. Accordingly, the absorbance values of nitrate and nitrite quantity in 19 composite soil samples were also measured and consequently, used to extrapolate the concentration of NO_3-N and NO_2-N in the soil and consequently, calculate the soil nutrient content present in each study site using the line equation derived from the standard calibration graphs. The standard line equations for NO_2^- and NO_3^- are shown in Figures 10 and 11 respectively and consequent concentrations of NO_3-N and NO_2-N were determined accordingly. A summary of NO_2^- and NO_3^- concentrations can be found in Table 11.

Table 8. The average absorbance values determined by UV-Vis spectrophotometry for known concentrations of NO_2-N standards

Concentration of NO_2-N (µg/l)	Avg. Absorbance
0	0.0005
10	0.033
20	0.065
50	0.162
100	0.323
250	0.857

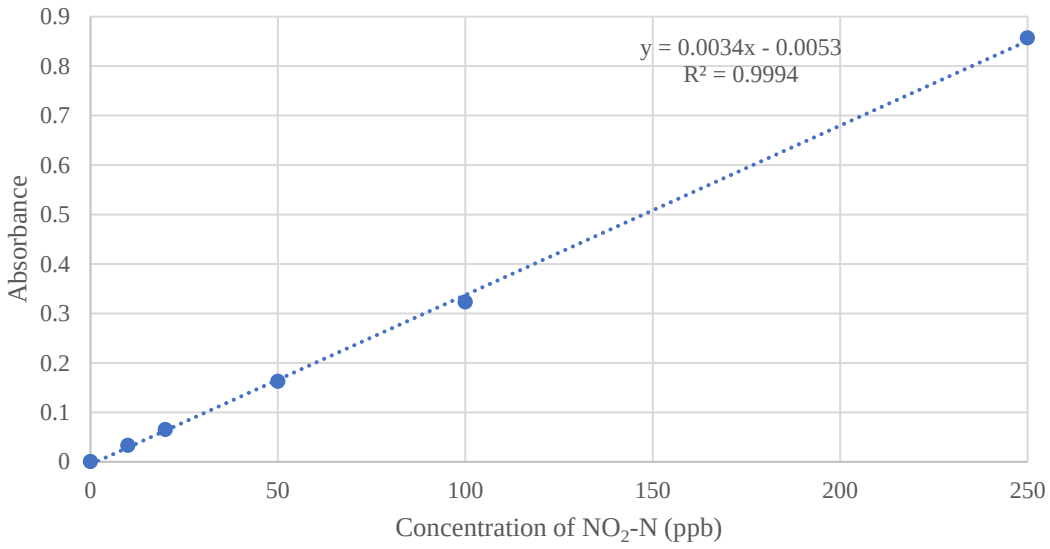

Figure 10. Standard calibration curve of known concentrations of NO_2-N

Table 9. The average absorbance values determined by UV-Vis spectrophotometry for known concentrations of NO_3-N standards

Concentration of NO_3-N (µg/l)	Avg. Absorbance
0	0.007
20	0.038
50	0.086
100	0.191
250	0.423

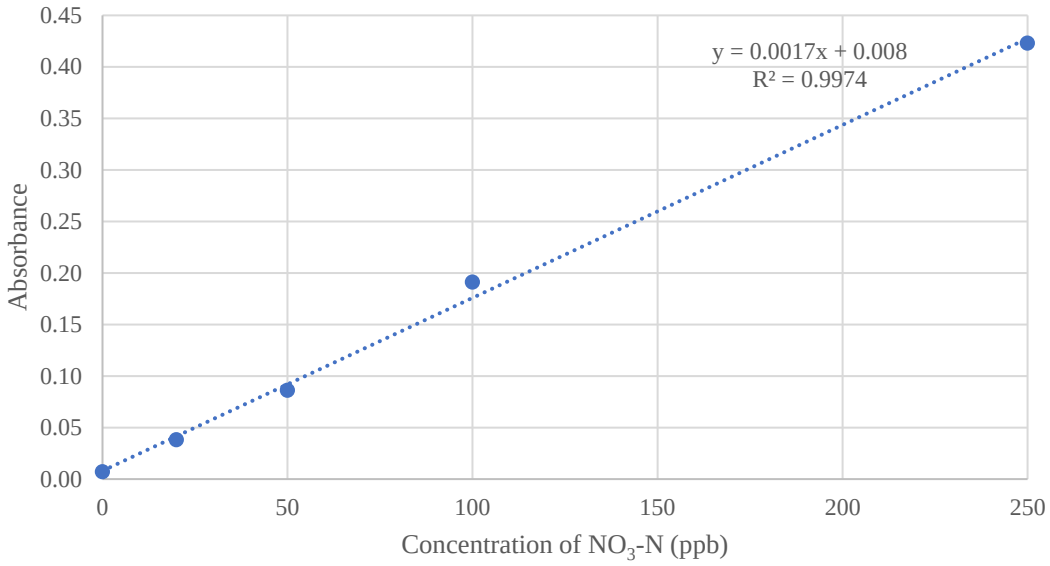

Figure 11. Standard calibration curve of known concentrations of NO_3-N

The data of each chemical variable measured in this study is summarised in Table 11. Shapiro-Wilk (S-W) normality test showed that the datasets for EC, NO_3^- and TC were normally distributed at 0.05 significance level (Appendix E). Variables that did not follow a normal distribution (sig. values < 0.05) were normalized by logarithmic transformation prior to ANOVA. Results of one-way ANOVA between locations and within each location is shown in Table 10. The significance (i.e. p-values) between the variable means is less than 0.05 alpha level and therefore, indicates that the chemical component differed significantly between locations. Consequently, Tukey HSD comparison was used to assess the significant differences between sample groups at 95% confidence interval. The results from Tukey comparison is presented in Table 11; values with similar letters were not significantly different between sample locations. Among the basic chemical parameters (that is, pH, salinity, EC and TDS) soil pH varied the least among the sites sampled in this study. The slight alkalinity in certain study sites was likely attributed to the large extent of inland saltmarshes, mangroves and surrounding coastal areas (site descriptions in Table 1). However, it should also be noted that the level of alkalinity shown in AlKhor mangroves and AlThakira saltmarsh were not the highest among the 19 study sites, as one would expect it to be. This, therefore, suggests that the degree of soil pH in each habitat or sampling site may not be the best indicator of soil quality and consequently, the fungal diversity and abundance in the respective environment.

Soil pH produced a more constant trend compared to the fluctuating salinity levels across the study sites. However, the trend in EC, TDS and salinity were more consistent as soil samples that had high salinity values also showed high EC. Given the similar trend in EC, TDS and salinity of soil extract, we may assume that these chemical characteristics were strongly influenced by Qatar's landform. The high EC and salinity

levels measured in soil extracts from AlKhor mangrove and AlThakira saltmarsh suggest highly saline depression soils that are likely dominated by halophytic plant species and soil microbiota. Additionally, the spike in EC and salinity at QU field, AlKharrara and AlRayyan study sites are likely the result of coastal and inland salt flats consisting of fine silt and high salt content (Abulfatih et al., 2002). Much of Qatar's central peninsula is composed of a plateau of limestone and sandstone outcrops, thereby contributing to the saline nature of the soil environment (Babikir, 1990). The results attained strongly suggest that the overall salinity of Qatar's soil habitats are highly saline.

Among the 19 study sites, AlKhor mangrove (i.e. wetland habitat) presented the highest salinity. TDS of soil extracts measured in different regions in Qatar suggests a certain extent of variation in soil chemical conditions and thus, may also imply differences in fungal communities between various land types across the country. It is also evident that regions of high salinity are most prevalent along coastal regions, lowlands and areas of swamps and saltmarshes. However, much of Qatar's topography is characterized by low-level flat lands; highly saline soils are thus, likely attributed to the intrusion of marine seawater into the groundwater across Qatar's peninsula. According to (Abulfatih et al., 2002), increasing agricultural practices and chemical industries has led to an increase in groundwater consumption. Given the highly saline soil conditions, halophytes are generally the favoured plant type in natural and human-induced plantation areas. However, combined with the rapid increase in anthropogenic disturbances, much of the natural soil conditions have been altered, thereby affecting the community structure of soil fungi.

The total carbon (TC) content determined in the composite soils fluctuated between locations while total nitrogen (TN) remained significantly low as %TN was

less than 1% in all the samples. The lowest and highest %TC values measured were found in samples from Dukhan (2.43 ± 0.01) and AlKharrara (10.99 ± 0.11). Samples collected from Dukhan area were distinct for its light sandy soil and abundant gravel debris. Most of Dukhan region lacked vegetation as the most dominant plant species existed in patches. Dukhan falls in the Western coast in Qatar and spans over an approximate area of 80 km. Dukhan is predominantly known for scattered regions of rawdha, soil depressions, and rock formations with various heights from the ground level. Given the topographic nature of Dukhan area, the low TC content measured in Dukhan's soils was expected as the area is characterized by major dry riverbeds that extends throughout the region. Moreover, given the lack of natural vegetation, agriculture and wildlife in the area, Dukhan may be deemed as unhabitable as the barren peninsula is eminent for extreme environmental conditions. As a result, the hard top soil layer in Dukhan does not support much natural plantation and microbial communities, thereby resulting in little soil nutrients.

On the other hand, the highest TC content measured in AlKharrara (i.e. lowland area) was predominantly dominated by regions of shrubs and thriving marine life (refer to Table 1 for site descriptions). Given the relatively high salinity in vegetation lands, a large extent of the plants inhabiting these areas are highly adapted to extreme environmental conditions. Additionally, plants found in wetlands or coastal habitats have the ability to mitigate the atmospheric carbon levels by sequestering carbon dioxide within plant roots beneath the soil surface. In this regard, the high TC content evident in soils sampled from Qatar's major mangrove regions was expected. Present halophytes in saline areas, saltmarshes and coastal regions were attributed to unique fungal communities and symbiotic arbuscular fungi adapted to the topography and extreme soil conditions.

In the case of TN concentrations, the data achieved indicated no general pattern across all the study sites as TN values fluctuated between samples. Majority of soil TN measured in each composite sample was negligible since the concentration of TN determined was well below the limits of detection. The only measurable soil TN content was determined in samples taken from AlKhor mangroves, although the TN value calculated was close to 0 (0.014% in AlKhor soil sample). Due to the significantly low TN concentrations shown in all the study sites, TN variable was excluded in subsequent statistical analyses. The undetectable concentrations of soil TN may be attributed to insufficient decomposition of soil organic matter, thereby resulting in poor nitrogen content in the soils (Prusty et al., 2009).

Table 10. Significance of the means between locations and within each location based on analysis of variance (ANOVA). *Means are statistically significant at 0.05 level (p-value < 0.05)

Source of Variance	df	Mean Square						
		pH	EC	Salinity	TDS	NO_2^-	NO_3^-	TC
Locations	18	0.412*	31492902.258*	10.457*	21.286*	9.161*	3581.790*	21.011*
Error	38	0.020	8.320	0.005	0.026	2.017	16.537	0.049
Total	56							

Table 11. Summary data of the soil properties at 19 sites locations Qatar. The values are displayed as the mean ± standard error (n = 3). Different letters indicate mean values with significant differences at 95% confidence interval, as determined by Tukey method

Location	pH	EC (µS/cm)	Salinity (psu)	TDS (g/l)	NO_2^- (mg/kg)	NO_3^- (mg/kg)	%TC	%TN
AlSakhama	7.62 ± 0.03^{e}	210.90 ± 0.52^{kl}	0.13 ± 0.03^{g}	0.14 ± 0.02^{f}	38.47 ± 0.37^{ab}	61.54 ± 0.36^{i}	5.15 ± 0.07^{e}	<DL
AlKhor	8.32 ± 0.07^{d}	9419.97 ± 0.55^{a}	5.43 ± 0.07^{a}	7.25 ± 0.07^{a}	36.36 ± 0.36^{abc}	73.71 ± 1.16^{ghi}	10.99 ± 0.11^{a}	0.01
AlThakira	8.95 ± 0.02^{a}	8099.67 ± 0.88^{b}	4.77 ± 0.03^{b}	6.74 ± 0.10^{b}	38.09 ± 0.56^{abc}	133.86 ± 0.08^{b}	9.18 ± 0.05^{b}	<DL
Lusail	8.80 ± 0.01^{abc}	449.27 ± 5.52^{g}	0.20 ± 0.00^{g}	0.70 ± 0.01^{e}	36.38 ± 0.48^{abc}	111.87 ± 6.17^{c}	6.19 ± 0.20^{d}	<DL
QU1	8.70 ± 0.10^{abcd}	637.73 ± 1.51^{f}	0.43 ± 0.03^{f}	0.70 ± 0.01^{e}	34.84 ± 0.57^{abcd}	88.14 ± 2.59^{def}	6.28 ± 0.31^{d}	<DL
QU2	8.43 ± 0.12^{cd}	7660.33 ± 1.45^{c}	4.47 ± 0.03^{c}	6.67 ± 0.24^{b}	34.04 ± 0.82^{cd}	98.14 ± 0.59^{d}	7.01 ± 0.07^{c}	<DL
AlGhuwayriyah	8.49 ± 0.03^{bcd}	175.63 ± 0.66^{mn}	0.13 ± 0.03^{g}	0.14 ± 0.02^{f}	34.95 ± 1.42^{abcd}	72.27 ± 0.55^{ghi}	4.32 ± 0.11^{f}	<DL
AlNu'man	8.30 ± 0.06^{d}	177.83 ± 0.26^{mn}	0.10 ± 0.00^{g}	0.16 ± 0.02^{f}	39.18 ± 0.52^{a}	95.42 ± 0.87^{de}	4.19 ± 0.09^{fg}	<DL
AlJumayliyah	9.00 ± 0.06^{a}	275.27 ± 1.16^{j}	0.13 ± 0.03^{g}	0.25 ± 0.02^{ef}	31.83 ± 0.83^{d}	91.07 ± 0.61^{de}	3.21 ± 0.01^{hi}	<DL
Khawzan	8.92 ± 0.03^{ab}	214.63 ± 2.01^{kl}	0.13 ± 0.03^{g}	0.22 ± 0.03^{ef}	36.24 ± 0.69^{abc}	83.88 ± 0.67^{efg}	2.64 ± 0.31^{ij}	<DL
AlKharrara1	8.71 ± 0.11^{abcd}	182.57 ± 0.62^{m}	0.10 ± 0.00^{g}	0.17 ± 0.03^{f}	38.10 ± 0.61^{abc}	72.71 ± 1.19^{ghi}	5.25 ± 0.11^{e}	<DL
AlKharrara2	8.50 ± 0.25^{bcd}	6370.33 ± 0.88^{d}	3.50 ± 0.06^{d}	5.42 ± 0.16^{c}	35.32 ± 0.88^{abcd}	44.21 ± 2.24^{j}	10.96 ± 0.06^{a}	<DL
AlRayyan	9.07 ± 0.06^{a}	3830.13 ± 0.59^{e}	2.13 ± 0.09^{e}	3.60 ± 0.25^{d}	35.38 ± 0.38^{abcd}	63.86 ± 0.48^{hi}	9.20 ± 0.03^{b}	<DL
AlKharsaah1	9.01 ± 0.01^{a}	363.93 ± 1.32^{h}	0.23 ± 0.03^{fg}	0.22 ± 0.03^{ef}	35.39 ± 0.28^{abcd}	111.15 ± 5.30^{c}	6.23 ± 0.07^{d}	<DL
AlKharsaah2	8.73 ± 0.07^{abcd}	218.70 ± 0.36^{k}	0.13 ± 0.03^{g}	0.18 ± 0.02^{f}	36.80 ± 0.92^{abc}	135.80 ± 2.97^{b}	4.16 ± 0.03^{fg}	<DL
Dukhan	9.06 ± 0.03^{a}	171.83 ± 0.82^{n}	0.10 ± 0.00^{g}	0.18 ± 0.02^{f}	34.64 ± 0.38^{bcd}	75.91 ± 0.35^{fgh}	2.43 ± 0.01^{j}	<DL
North Qatar1	8.99 ± 0.01^{a}	206.47 ± 0.29^{l}	0.17 ± 0.07^{g}	0.19 ± 0.01^{f}	37.00 ± 0.85^{abc}	165.18 ± 1.03^{a}	3.59 ± 0.10^{gh}	<DL
North Qatar2	9.03 ± 0.01^{a}	155.30 ± 2.03^{o}	0.13 ± 0.03^{g}	0.17 ± 0.02^{f}	36.94 ± 1.88^{abc}	114.65 ± 3.23^{c}	4.08 ± 0.09^{fg}	<DL
AlKiranah	8.42 ± 0.03^{cd}	301.10 ± 1.50^{i}	0.10 ± 0.00^{g}	0.25 ± 0.02^{ef}	36.89 ± 0.95^{abc}	15.98 ± 1.00^{k}	3.93 ± 0.18^{fg}	<DL
Total	8.69 ± 0.05	2059.03 ± 421.42	1.19 ± 0.24	1.75 ± 0.35	36.15 ± 0.28	89.97 ± 4.52	5.74 ± 0.35	

4.1.3 Trace Metal Concentrations by ICP-OES

The concentrations of six trace metals: Ca, Cd, K, Mg, P and Pb were determined by ICP-OES. Among the tested components, Ca content in the soils showed the highest concentrations as values were well over 90,000 mg/kg in all the locations. On the other hand, the lowest measured concentrations were derived from the Cd metal as Cd values ranged from as low as 0.02 mg/kg to 0.40 mg/kg. Quality control in the measurements achieved from ICP-OES was ensured by including certified reference materials (PACS3-1 and PACS3-2); subsequent %R of each metal fell within 15% of EPA criteria (Table 12). Table 13 summarizes the metal concentrations measured at each location.

Table 12. The metal concentrations of certified reference materials (PACS3-1 and PACS3-2) were determined to ensure quality control and reliability in the readings achieved from ICP-OES

	PACS 3-1	PACS 3-2	PACS 3-1	PACS 3-2	PACS 3-1	PACS 3-2	Avg.	Cert. Conc.	%R
Ca	18086	17625	18503	18016	18770	18897	18316	18900	97
Cd	2.37	2.24	2.36	2.36	2.36	2.48	2.4	2.23	106
K	12891	12625	12019	12395	12149	11791	12312	12530	98
Mg	13848	13414	14008	13700	14017	14470	13909	14020	99
P	852	876	846	866	848	873	860	937	92
Pb	165.7	169.7	166.5	171.8	168.7	172.6	169	188	90

Table 13. Concentration of soil macronutrients (Ca, K, Mg, P) and heavy metals (Cd, Pb) measured at different sampling-sites. The average concentrations were taken of each trace metal was determined in composite samples from each location

Location	Concentration (mg/kg)					
	Ca	Cd	K	Mg	P	Pb
AlSakhama	141175	0.4	9163	30090	522	8.67
AlKhor	286904	0.11	2393	15153	319	1.7
AlThakira	294279	0.08	2023	20633	269	2.08
Lusail	165214	0.19	6483	26043	305	4.46
QU1	151710	0.29	6250	29404	345	15.29
AlGhuwayriyah	108471	0.35	11713	40469	678	7.32
AlNu'man	101232	0.36	11103	38031	736	9.76
AlJumayliyah	95174	0.27	8592	10271	133	3.18
Khawzan	92467	0.19	7381	14302	140	3.41
QU2	173927	0.07	5542	22595	262	6.35
AlKharrara1	138876	0.27	9046	28272	478	7.34
AlKharrara2	295909	0.1	2355	15426	336	1.4
AlRayyan	276665	0.02	2790	21854	255	0.12
AlKharsaah1	172888	0.22	6485	25924	266	3.4
AlKharsaah2	107655	0.2	8795	19531	278	4.76
Dukhan	74949	0.15	6104	7334	123	2.95
North Qatar1	98698	0.18	8006	15430	173	3.2
North Qatar2	108095	0.14	7617	17552	160	2.96
AlKiranah	100426	0.31	9741	25062	345	5.18

4.1.4 Correlation between Chemical Variables and Trace Metals

Pearson correlation matrix was computed to evaluate the covariance between the soil chemical variables. According to Pearson correlation matrix, increasing pH values was negatively correlated with increasing EC (-0.020), salinity (-0.023), TDS (-0.015), TC (-0.513) and NO_2^- concentration (-0.513). The correlation between pH and these variables were not statistically significant at 0.05 alpha level. pH also indicated a small positive correlation with soil NO_3^- (0.010) (Table 14). Additionally, a strong

positive correlation is seen between EC, salinity and TDS, where the r values equalled to 1 (Table 14). In this case, either of these three variables may be removed in subsequent analysis as it does not affect the quality of the results achieved.

Table 14. Pearson correlation coefficient matrix of soil chemical parameters (N = 7)

Variables	EC	Salinity	TDS	TC	Nitrite	Nitrate
pH	-0.020	-0.023	-0.015	-0.056	-0.513	0.010
EC		**1.000**	**1.000**	**0.845**	-0.157	0.405
Salinity			**0.999**	**0.840**	-0.150	0.405
TDS				**0.844**	-0.162	0.413
TC					0.008	0.087
Nitrite						-0.304

Values in bold are significant at 0.05 alpha level

Following Pearson coefficient analysis, a principle components analysis (PCA) between the seven chemical components was carried out to analyse potential correlations or variations between these variables and subsequently, identify uncorrelated factors. Eigen values were computed to assess the cumulative variability in chemical data. The first and second eigen values (3.953 and 1.575 respectively) combined represents approximately 79% of the total variability (Table 15). This is also evident in the scree plot as the first two factorial components (F1-F7) are sorted in descending order to indicate the cumulative variability of the data (Table 15). Although the initial PC test incorporated all seven factorial components, the last two factors (F6 and F7) were discarded in the subsequent computation of the eigen vectors since 99.99% of the variability has been accounted for in F5. In other words, factors 6 and 7 were not correlated as the r value was close to 0 (Table 15). The two-dimensional map indicated two distinct groups of locations that were influenced by specific chemical parameters while sampled areas such as Dukhan beach showed that it had no correlation

with any of the chemical components. It is evident that the demographics of QU field are unique in a way that it is not directly influenced by the basic soil chemistry (Figure 12). We could also confirm the soils in AlSakhama had the highest nitrite content compared to other sampled areas while soil samples collected from Dukhan and AlJumayliyah had the highest measured pH.

Table 15. Eigen values extrapolated from PCA computation of chemical parameters

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	3.953	1.575	0.922	0.409	0.141	0.001	0.000
Variability (%)	56.469	22.506	13.169	5.838	2.011	0.007	0.001
Cumulative (%)	56.469	78.974	92.143	97.981	99.992	99.999	100.000

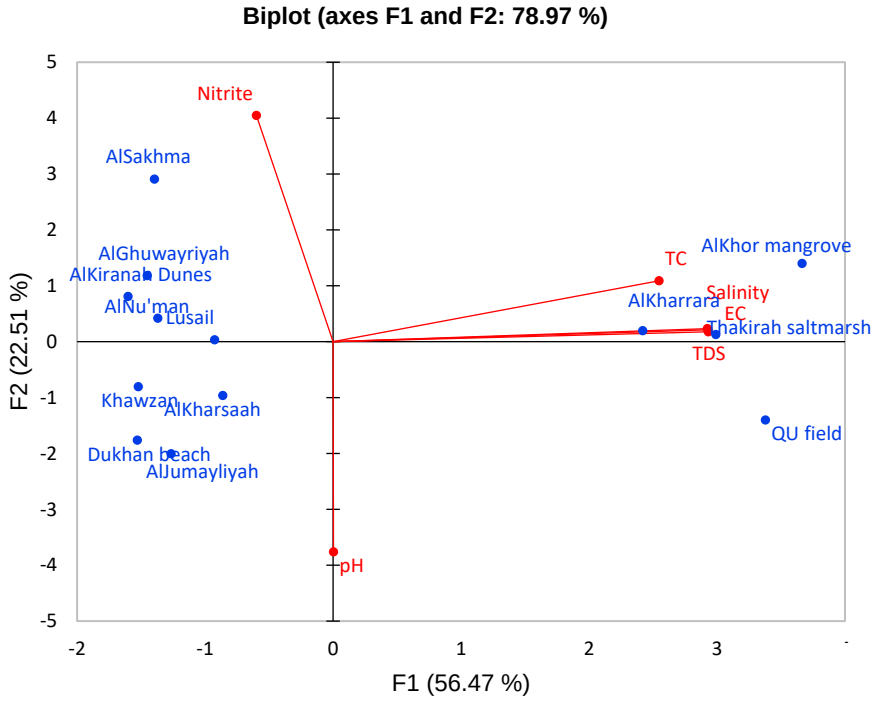

Figure 12. Two-dimensional map indicating the correlation trend between sampled locations and variability of the chemical parameters. The study areas are shown in blue while the variability of chemical components is displayed by the red lines

Given the negative r values computed by Pearson correlation, Ca was negatively correlated with the five other metals measured in this study: Cd (-0.693), K (-0.838), Mg (-0.082), P (-0.087) and Pb (-0.461), which suggest that Ca content in the soils are highly independent (Table 16). Excluding Ca content from the analysis would not have affected the concentration of the remaining metals and therefore, could have easily been removed without affecting the quality of the results achieved. However, the remaining metals, Cd, K, Mg, P and Pb showed positive correlations which in turn, could potentially influence the diversity and distribution of AMF communities. According to Pearson coefficient matrix, the trace metals, Cd is mostly positively correlated with K (0.926) while Pb is mostly positively correlated with Cd (0.722).

Table 16. Pearson correlation coefficient matrix of tested trace metals (N = 6)

Variables	Cd	K	Mg	P	Pb
Ca	**-0.693**	**-0.838**	-0.082	-0.087	-0.461
Cd		**0.926**	**0.573**	**0.639**	**0.722**
K			**0.544**	**0.556**	**0.647**
Mg				**0.916**	**0.677**
P					**0.649**

Values in bold are different from 0 with a significance alpha level of 0.05

PCA was carried out to assess the variation between trace metal concentrations. PC analysis of the trace metals computed eigen values that reflect the quality of variables from the initial N-dimensional (N = 6) to a lower number of dimensions. The first eigen value was found to be 4.061 which represented approximately 68% of the total variability (Table 17). In other words, the majority of the percentage variability in the data achieved may be presented by the first dimension, F1. Additionally, the first and second factors (that is, F1 and F2) combined represent approximately 91% of the

total variability in the data (Table 17). This high % variance ensures that further PC analysis based on the first two factors produces a sufficient projection of the initial multi-dimensional table. The correlation circle computed from PCA projects the variability of factors in space. F1 and F2 accounts for 67.68% and 22.95% of the variability in trace metals respectively (Figure 13). The concentrations of Mg and P metals are significantly positively correlated (r value is close to 1), while Ca is significantly negatively correlated (r value is close to 0). The horizontal axis (F1) is linked with five out of the six trace metals (Mg, P, K, Pb and Cd) determined in this study, while the vertical axis (F2) is associated with only Ca content.

Table 17. Eigen values computed from PC analysis of trace metals

	F1	F2	F3	F4	F5	F6
Eigenvalue	4.061	1.377	0.344	0.146	0.064	0.009
Variability (%)	67.684	22.948	5.727	2.425	1.061	0.155
Cumulative (%)	67.684	90.632	96.359	98.784	99.845	100.000

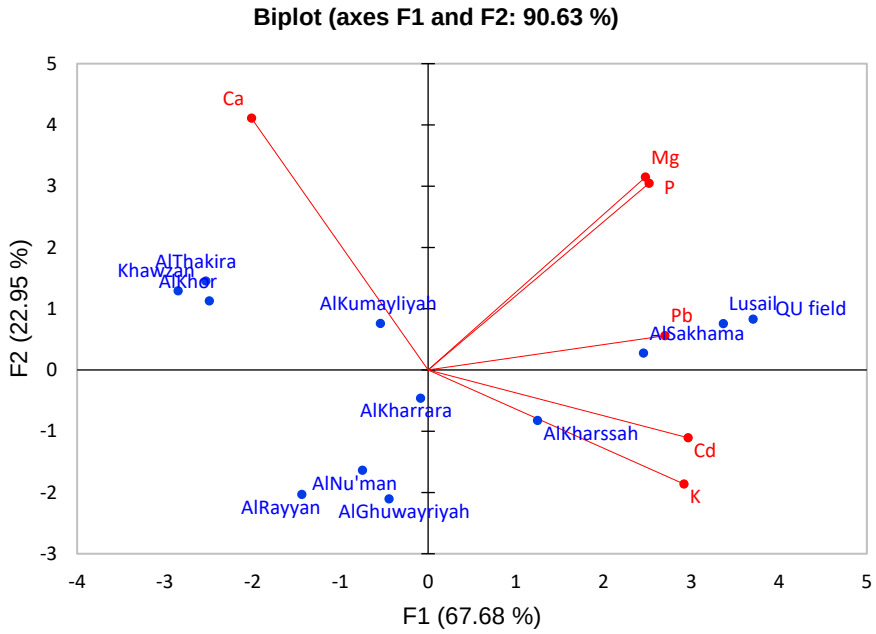

Figure 13. Correlation circle computed from PCA to assess the variability of trace metals

4.2 PCR Amplification

To analyze the fungal communities within the soil environment, PCR amplification was used to target the SSU region of rRNA gene. Tagged universal eukaryotic primer WANDA was paired with a Glomeromycota-specific primer AML2 to produce amplicons that were approximately ~ 500 bp in length (Figure 14). Quantification of PCR products were estimated by 1.5% agarose gel electrophoresis. Results indicated by agarose gel electrophoresis indicated that DNA extracts from all composite samples could be amplified by PCR, however, the strength of amplification of DNA extracts from sites 13 and 14 subsamples were lower than those that have been mixed and homogenized (that is, the composite soil samples). Based on the strength of the gel band, the PCR program was optimized by increasing the number of cycles per run while maintaining the program set for denaturation, annealing and extension (refer to Section 3.3.2 of the Materials and Methods). PCR amplification was repeated using DNA extracts from the composite sample of site 18 and subsamples of sites 13 and 14 for 38 cycles.

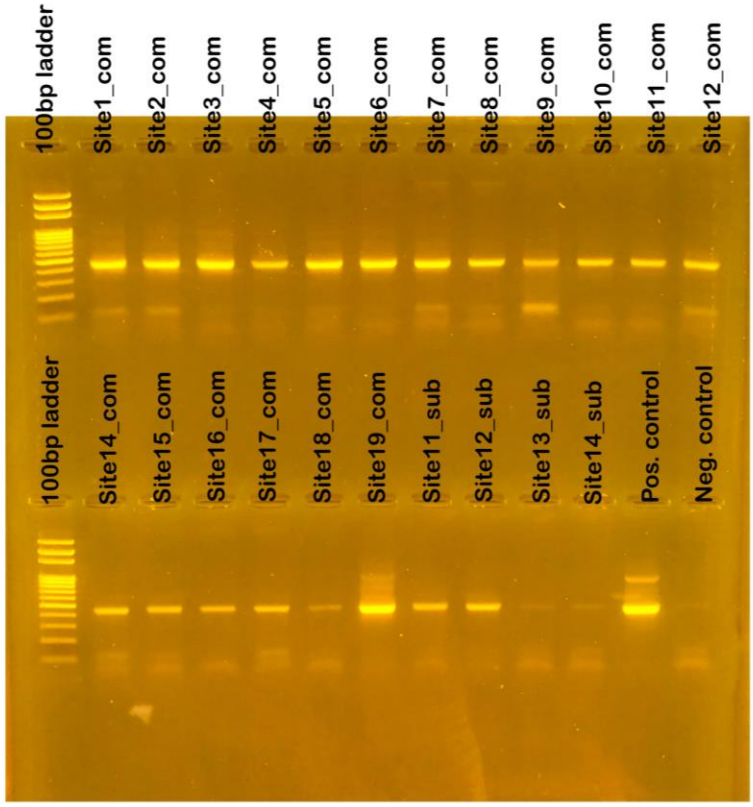

Figure 14. Sybr-safe stained agarose gel electrophoresis (1.5%) image of 5 µl PCR products using tagged WANDA and AML2 primer pairs. 3 µl of DNA ladder was injected in the first lanes to indicate the approximate length of DNA amplicon (~500 bp). Positive (AMF from the roots of *Medicago sativa*) and negative control (PCR excluding a template DNA) are shown in the last two lanes of the gel to ensure that there was no possibility of contaminants during the PCR process

4.3 Fungi Sequencing Results

4.3.1 Diversity Data and Relative Abundance

Illumina 2x300 bp MiSeq platform generated 2x9,861,189 paired-end reads targeting the SSU region of the rRNA gene. A total of 2,474,899 sequences were quality filtered and combined, of which, 3.3% were determined as putative chimeric reads. Following the removal of these chimeric reads, positive control sample and singletons (6 singletons), the final dataset comprised of 53 VTs, 1,369,477 sequences and 112 samples. Each sequenced sample comprised of 2 to 62,040 sequences (median: 6588 sequences) and 1 to 28 VTs (median: 7 VTs). Table 18 summarizes the number of sequence reads following each step of the bioinformatics analysis (as described by Vasar et al., 2017).

Table 18. Sequencing (Illumina MiSeq) outputs from different bioinformatics steps

Steps	Remaining number of reads
Targeted marker region	Small subunit (SSU) ribosomal RNA gene V4 region
Primers	F: WANDA (Dumbrell et al., 2011); R: AML2 (Lee et al., 2008)
Raw reads	2x9,861,189
Quality filtering of sequences with adapter contamination (cleaned barcode and primer)	5,200,956
Combining quality-filtered paired-end reads	2,474,899
98% clustering	614,884
Chimera filtering	594,894
BLAST search with 97% identity and 95% alignment against MaarjAM database (without positive sample and singletons)	53 VT (1,368,477 reads)
BLAST search with 85% identity and 95% alignment against MaarjAM database (without positive sample and singletons)	127 VT (1,788,424 reads)

Results on the diversity of AMF families, relative abundance and population of AMF in the study areas are presented in Figures 15 and 16, respectively. Figure 15 indicates the relative abundance and distribution of eight identified AMF families in the study regions. The results attained from BLAST search at 97% and 85% identity level against MaarjAM database reference are displayed in Figures 15a and 15b, respectively. AMF families identified at 97% identity include *Glomeraceae*, *Claroideoglomeraceae*, *Diversisporaceae* and *Paraglomeraceae*. The dataset in Figure 15a shows that 83% of the AMF population is from the family *Glomeraceae* while 15% is from the family *Claroideoglomeraceae*. These results suggest that *Glomeraceae* is the dominant family group thriving in Qatar's extreme habitat. However, the evenness of AMF families among the sites are not evenly distributed as several regions including Lusail, AlJumayliyah and AlKiranah dunes are largely populated by *Claroideoglomeraceae*; suggesting that specific AMF species respond differently to various environmental conditions that allow them to successfully occupy the region.

Alternatively, the number of AMF species established from BLAST search at 85% identity level was higher compared to those identified at 97% identity level since 42% of sequences were lost when using the default threshold (i.e. 97% identity and 95% alignment). In addition to the four families identified at 97% identity, Figure 15b also indicates the existence of four additional family groups: *Acaulosporaceae*, *Archaeosporaceae*, *Ambisporaceae* and *Gigasporaceae*. However, the relative abundance of these four families were significantly smaller than families identified at 97% identity level (Figure 15a) and only occurred in two to four sites out of the 19 study sites. Due to the significantly small number of species from these four families, their abundance may be considered as negligible or redundant (less than 1% abundance in the pie chart of Figure 15b) since 76%, 13%, 6% and 5% of the relative abundances

in most sites are largely populated by *Glomeraceae*, *Claroideoglomeraceae*, *Paraglomeraceae* and *Diversisporaceae*, respectively. Compared to the dataset presented in Figure 15a, *Paraglomeraceae* and *Diversisporaceae* populations appear to be more abundant in several sites including AlKhor mangrove, AlThakira saltmarsh and Khawzan. The results displayed in Figure 15b indicates a more even distribution of AMF families among the study sites. However, higher abundance of certain AMF groups in specific sites also implies that these regions may be inhospitable for some species groups given its extreme environmental conditions, but may be better-suited for other AMF families as a result of natural adaptation.

The number of sequence reads generated from Illumina MiSeq in the soil samples collected from all the samples ranged from 48 to 75912. The number of VTs for each of AMF family ranged from 1 to 87 with a total of 127 VTs and an average AMF population of 15.9. Determination of Shannon index revealed that *Glomeraceae* exhibited the highest species diversity (Shannon index of 0.468) among eight of the AMF families (Table 19). On the other hand, AMF families: *Ambisporaceae* and *Gigasporaceae*, indicated the lowest species diversity as the Shannon indices determined for both families (0.065 and 0.038 respectively) were close to zero (

Table 19). Taking into account the number of AMF species and relative population size of each fungal family (Figure 16), the high Shannon index value determined for *Glomeraceae* suggests that the family exhibited the highest species richness and evenness among the sites sampled. Additionally, values obtained for AMF diversity using Simpson indices were highest for *Glomeraceae* and lowest for *Ambisporaceae* and *Gigasporaceae*. Determination of Simpson diversity indices for each of the identified AMF families indicated a similar pattern achieved by Shannon indices and thus, further establishes the AMF family, *Glomeraceae,* as the dominant family across all the dataset. Detailed information on all AMF taxa identified in this study can be found in Appendix H.

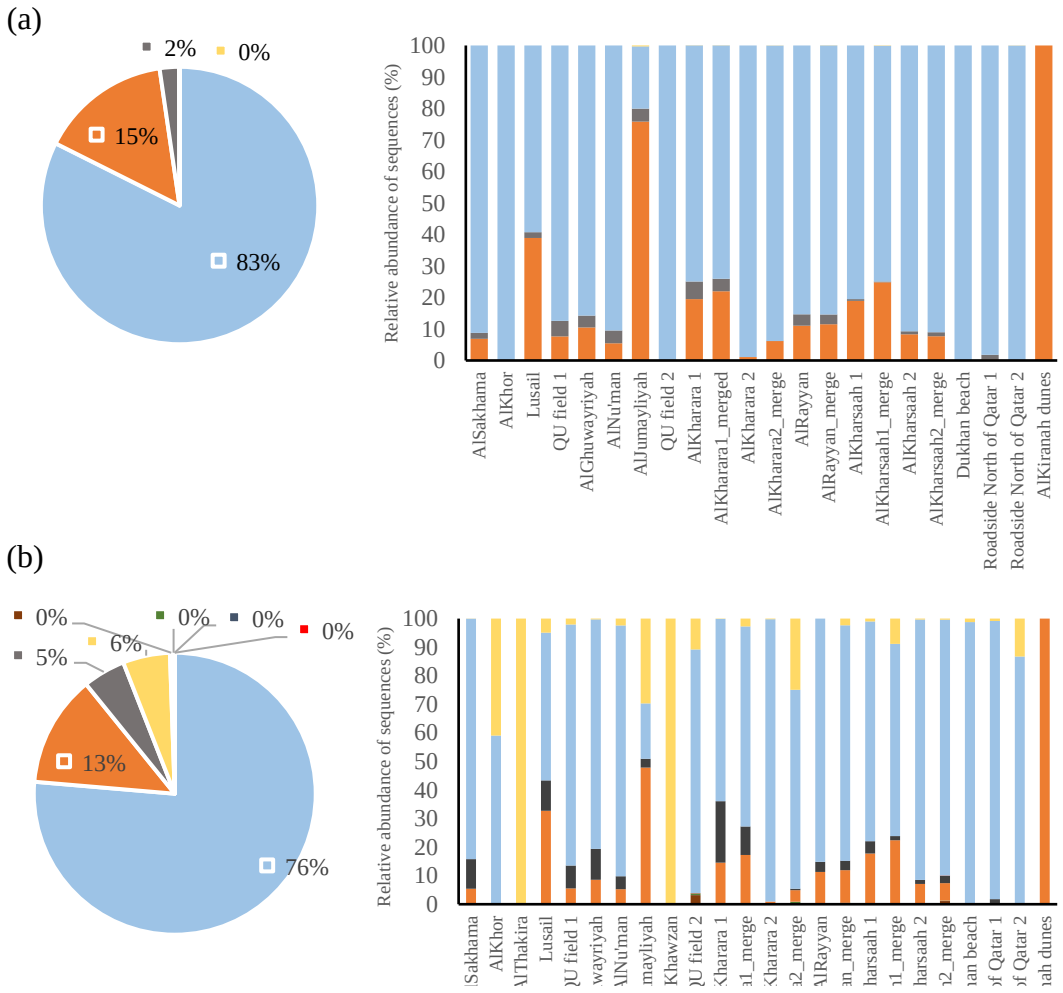

Figure 15. Relative abundance of AMF families presented across all dataset (pie chart) and per site (bar chart). AMF species are identified by running a BLAST search against MaarjAM database reference at (a) 97% identity level and 95% alignment threshold; (b) 85% identity level and 95% alignment threshold, which identified 42% more AMF sequences. The abundance of some families was less than 1%. AMF families are presented according to the following colours: *Acaulosporaceae* - , *Ambisporaceae* - , *Archaeosporaceae* - , *Claroideoglomeraceae* - , *Diversisporaceae* - , *Gigasporaceae* - , *Glomeraceae* - , *Paraglomeraceae* -

Table 19. Data on the sequence analysis, population of AMF and **α** biodiversity indices determined for eight different AMF families

Family	No. of sequences	No. of AMF VTs	Shannon index	Simpson index	Dominance index
Glomeraceae	1,365,588	87	0.259	0.468	0.532
Claroideoglomeraceae	229,080	7	0.160	0.003	0.997
Diversisporaceae	87,319	10	0.200	0.006	0.994
Paraglomeraceae	96,676	7	0.160	0.003	0.997
Acaulosporaceae	5415	6	0.144	0.002	0.998
Archaeosporaceae	4045	7	0.160	0.003	0.997
Ambisporaceae	200	2	0.065	0.000	1.000
Gigasporaceae	101	1	0.038	0.000	1.000
Total	1,788,424	127	1.186	0.483	7.517

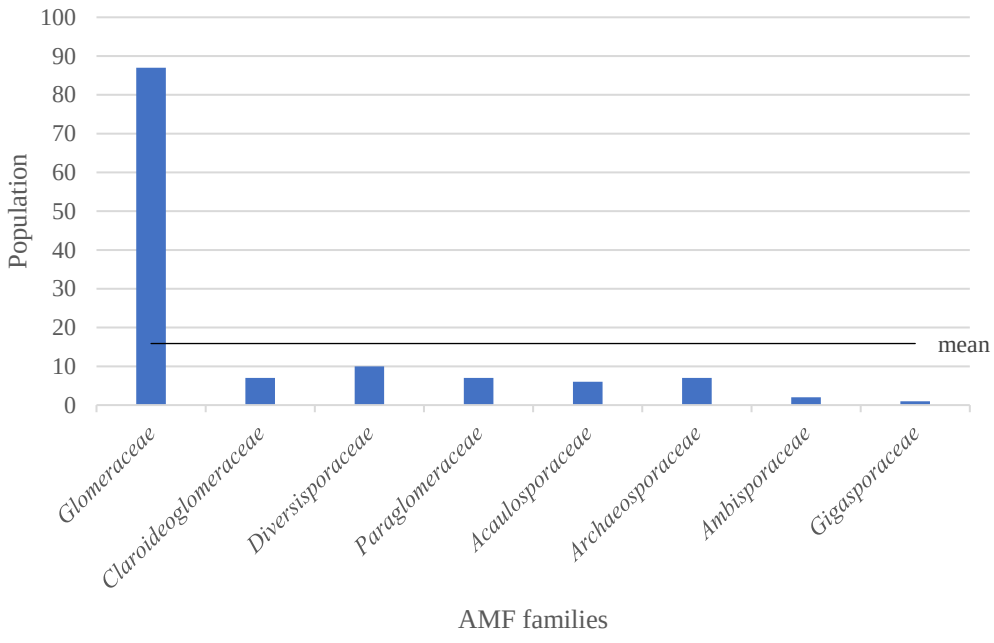

Figure 16. Population distribution of AMF families indicates *Glomeraceae* as the dominant AMF population group and *Gigasporaceae* as the lowest population

4.3.2 AMF Community Composition and Frequency of Occurrence

Identified AMF species were associated with eight AMF genera (*Acaulospora, Ambispora, Archeospora, Claroideoglomus, Diversispora, Scutellospora, Glomus* and *Paraglomus*). Species were identified according to sequences obtained from the AMF families: *Glomeraceae* (87 VTs), *Diversisporaceae* (10 VTs), *Claroideoglomeraceae* (7 VTs), *Paraglomeraceae* (7 VTs), *Archaeosporaceae* (7 VTs), *Acaulosporaceae* (6 VTs), *Ambisporaceae* (2 VTs) and *Gigasporaceae* (1 VT). Indicator species analysis revealed that AMF indicators originated from three main taxa: *Glomeraceae* in 15 sites, *Paraglomeraceae* in 2 sites and *Claroideoglomeraceae* in 2 sites (indicator index value > 0.25). However, indicator index value was highest for *Glomeraceae* (13.33) and comparatively equal for *Paraglomeraceae* (2.98) and *Claroideoglomeraceae* (2.35) (Appendix G). Using the identified AMF VTs, differences between soil AMF communities was determined by agglomerative hierarchical clustering (AHC), which classified the AMF in 19 soil samples into four classes, each representing different soil types (Figure 17).

Variation in the composition of AMF genera was observed among the soil samples collected from all study locations (Table 20). The highest frequency of occurrence in the sites sampled was recorded to be 80% which was recognized as species from *Glomus* genus and thus, were the dominant AMF taxa in majority of the study sites. Consequently, this was followed by the genera: *Paraglomus* (73%), *Claroideoglomus* (67%) and *Diversispora* (67%), while the percentage frequency of occurrence of the remaining AMF genera ranged between 13% to 27%. However, no one genus was identified in all the sampled locations as the frequency of occurrence determined for each AMF genus did not reach 100% occurrence. *Glomus* species was not recorded in three locations, that is, AlThakira saltmarsh, Khawzan and AlKiranah

sand dunes, while on the other hand, species from the genera *Acaulospora* and *Archeospora* were only recorded in two of the nineteen locations sampled. The infrequent occurrence of genera, *Acaulospora* and *Archeospora* could potentially imply its scarcity in highly arid regions which are often restricted in resources. The top 50 AMF VTs from each sampled site were used to compare their abundances. The data was presented as a heat map in Figure 18. More detailed information of all identified AMF VTs can be found in Appendix H.

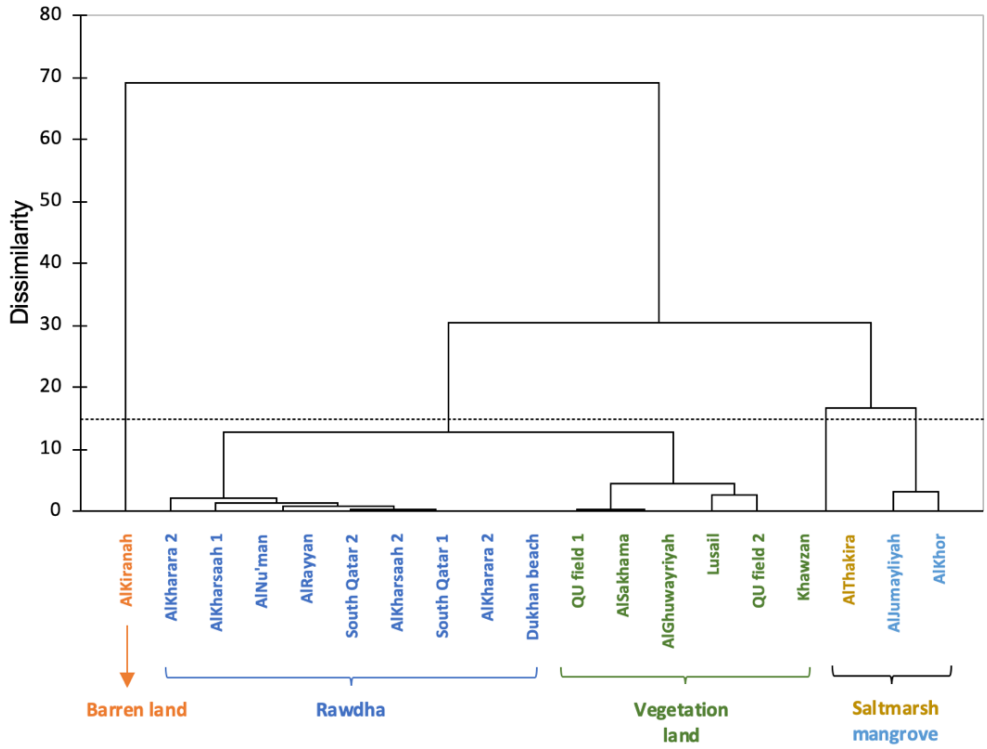

Figure 17. AMF clustering analysis based on the abundance of identified AMF families. The dendrogram computed by AHC shows four clusters, each indicating different soil types of AMF community composition

Table 20. Distribution of each identified AMF genus, frequency of occurrence (%) and richness in each study location

Location	Genus							
	Acaulospora	*Ambispora*	*Archaeospora*	*Claroideoglomus*	*Diversispora*	*Scutellospora*	*Glomus*	*Paraglomus*
AlSakhama	-	-	-	+	+	-	+	-
AlKhor	-	-	-	-	-	-	+	+
AlThakira	-	-	-	-	-	-	-	+
Lusail	-	-	-	+	+	-	+	+
QU field	+	-	+	+	+	+	+	+
Ghuwayriyah	-	-	-	+	+	-	+	-
AlNu'man	-	-	-	+	+	-	+	+
AlJumayliyah	-	-	-	+	+	-	+	+
Khawzan	-	-	-	-	-	-	-	+
AlKharrara	-	+	+	+	+	+	+	+
AlRayyan	-	+	-	+	+	+	+	-
AlKharsaah	+	+	-	+	+	+	+	+
Dukhan beach	-	-	-	-	-	-	+	+
South of Qatar	-	-	-	-	+	-	+	+
AlKiranah	-	-	-	+	-	-	-	-
Frequency of occurrence (%)	13	20	13	67	67	27	80	73

+: Present genus; -: Absent genus

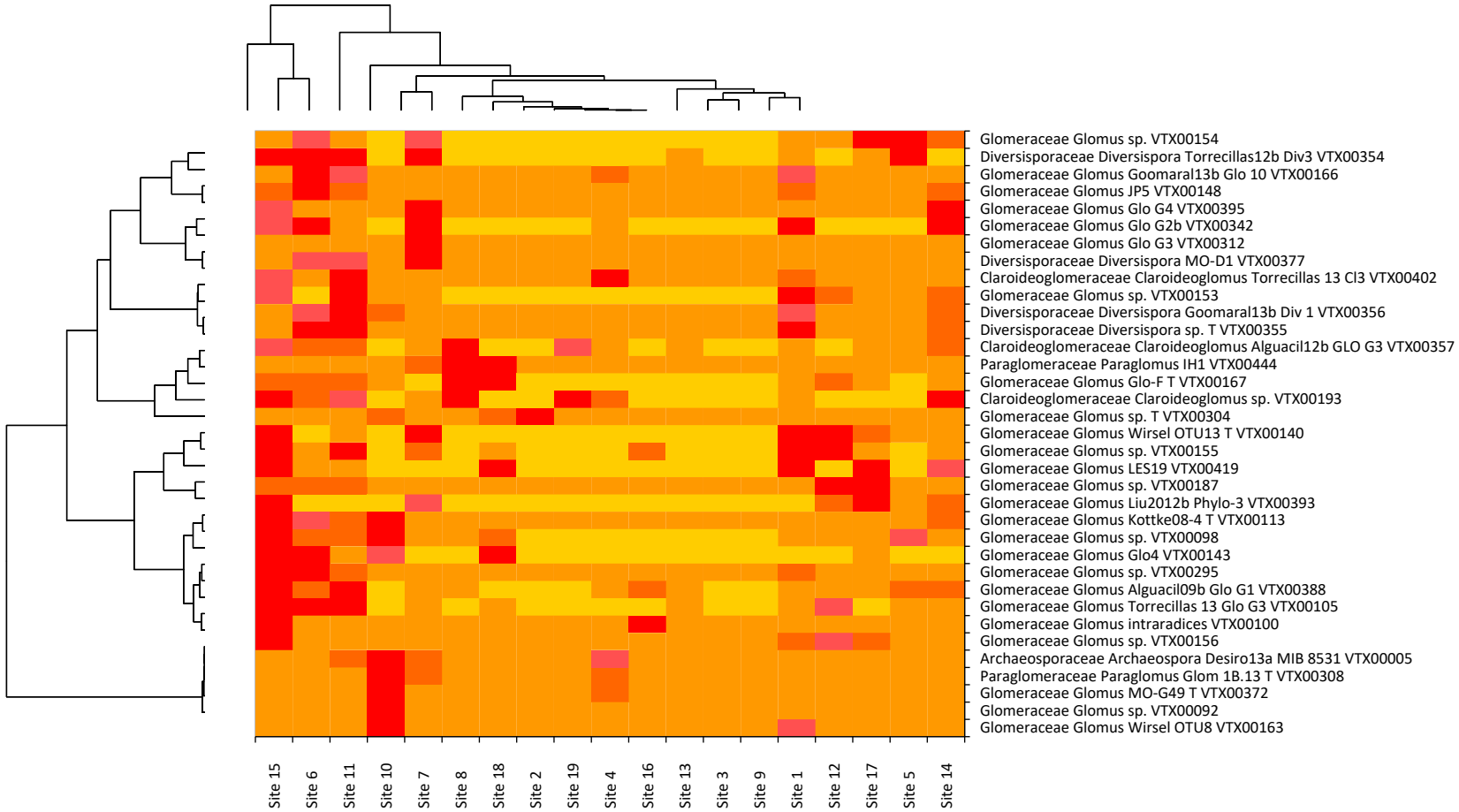

Figure 18. Heat map of AMF VTs identified from all study sites. The intensity of the colour corresponds to the abundance of AMF species present in each sample (the highest to lowest abundance is shown from red to orange to yellow)

4.4 Effect of Chemical Components and Land-type on AMF Distribution

To determine the extent of correlation between chemical parameters, land type and AMF community, a distance-based redundancy analysis (RDA) was carried out. The sites used in this study were classified into one of five main land-types: rawdha, saltmarsh, mangrove, barren land, and vegetation area. Approximately 61% of the variability in AMF community can be justified according to the environmental variables (pH, TC, salinity, EC, TDS and NO_3^-) and land type investigated in by RDA (Figure 19). The results derived from the redundancy test based on 1000 permutations can be used to verify whether the response variables (that is, AMF community) is linearly correlated to the explanatory variables (that is, chemical parameters and land type). Since the computed p-value (0.012) is less than alpha at 0.05, the null hypothesis is rejected (Table 21), thereby justifying that there is a significant linear correlation between the response (i.e. AMF distribution) and explanatory variables (i.e. chemical parameters). The risk of rejecting the null hypothesis while it is true is lower than 1.20%. Permutation test was carried out to prove the reliability of the subsequent redundancy analysis results.

Table 21. Results of the permutation test derived from redundancy analysis

Permutations	1000
Pseudo F	2.984
p-value	0.012
alpha	0.050

H_0: The chemical variables and land type are not linearly related to AMF
H_α: The chemical variables and land type are linearly related to AMF

The RDA plot indicates the relationship between 12 tested chemical factors and five different land types with the distribution of identified AMF genera. With axes F1

and F2 combined, around 61% of the constrained inertia or distribution of AMF is influenced by the chemical parameters and land types combined (Figure 19). Axis F1 indicated the greatest correlation with the explanatory variables: EC (0.095), salinity (0.092) and TDS (0.089), while the explanatory variables on Axis F2 was only positively related to soil pH (0.042) and VG land types (that is, locations indicating vast vegetation coverage, 0.290) and negatively related to the remaining environmental characteristics.

On the other hand, sites that were categorised as vegetation land-types were more closely linked to soil nitrate and pH. However, the overall effect of soil pH on AMF distribution was significantly lower compared to the relationship between AMF communities and nitrate. Sites with extensive vegetation coverage and higher nitrate content were mostly associated by AMF species from the genera: *Claroideoglomus* and *Scutellospora*. RDA plot also indicated that the effect of chemical parameters is much lower on *Glomus* species compared to other fungal phyla (Figure 19). As a result, *Glomus* species are more abundant and widely distributed in the study sites, likely due to their resilient nature towards environmental characteristics.

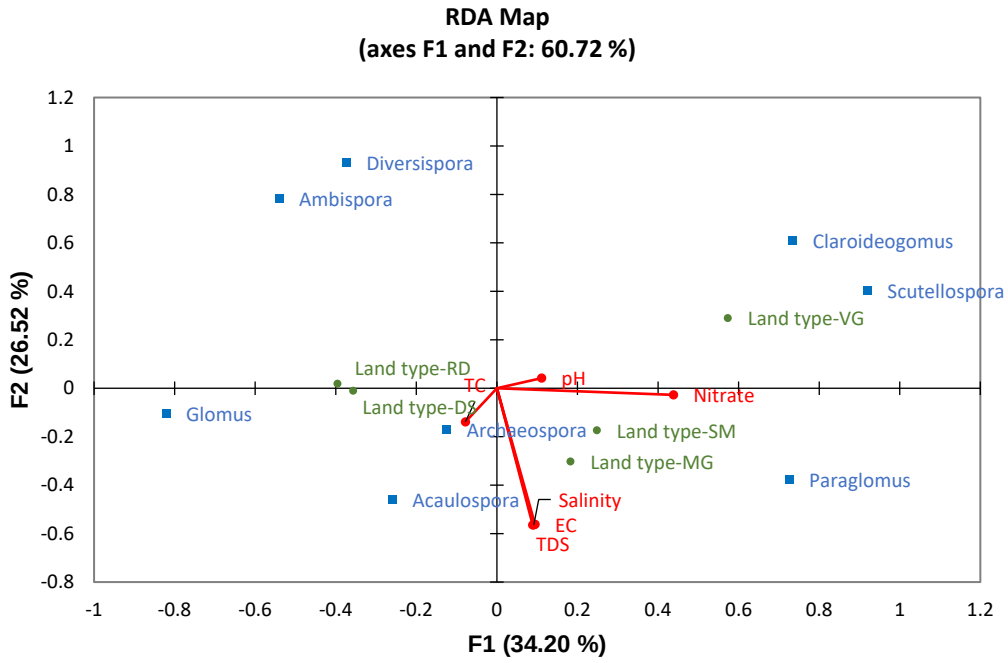

Figure 19. Distance-based redundancy plots used to interpret the extent of correlation between different land types and chemical variables with AMF communities. The land types used in RDA are listed as follows: vegetation/shrub land (VG), saltmarsh (SM), mangrove (MG), desert/barren land (DS) and rawdha (RD)

The RDA plot in Figure 20 presented the correlation between six trace metals and the distribution of AMF species. With axes F1 and F2 combined, the RDA map accounts for approximately 66% of the chemical variables (Figure 20). In other words, 66% of variation in AMF communities were attributed to present trace metals in the soil environment. According to the standardized coefficients computed by RDA, Axis F1 indicated positive correlations with the explanatory chemical variables: Mg (0.455), P (0.334), Pb (0.309), Ca (0.034) and K (0.033); all of which mostly affect the distribution of *Diversispora*, *Ambispora* and *Glomus*. However, soil Mg content showed the greatest effect on AMF distribution in Axis F1. On the other hand, the variation coefficients of Cd (0.478) and K (0.522) shown in Axis F2 attributed to most

of the correlation pertaining AMF distribution. Accordingly, it is also apparent that Cd and K had the most influence on the distribution of *Diversispora* and *Ambispora*.

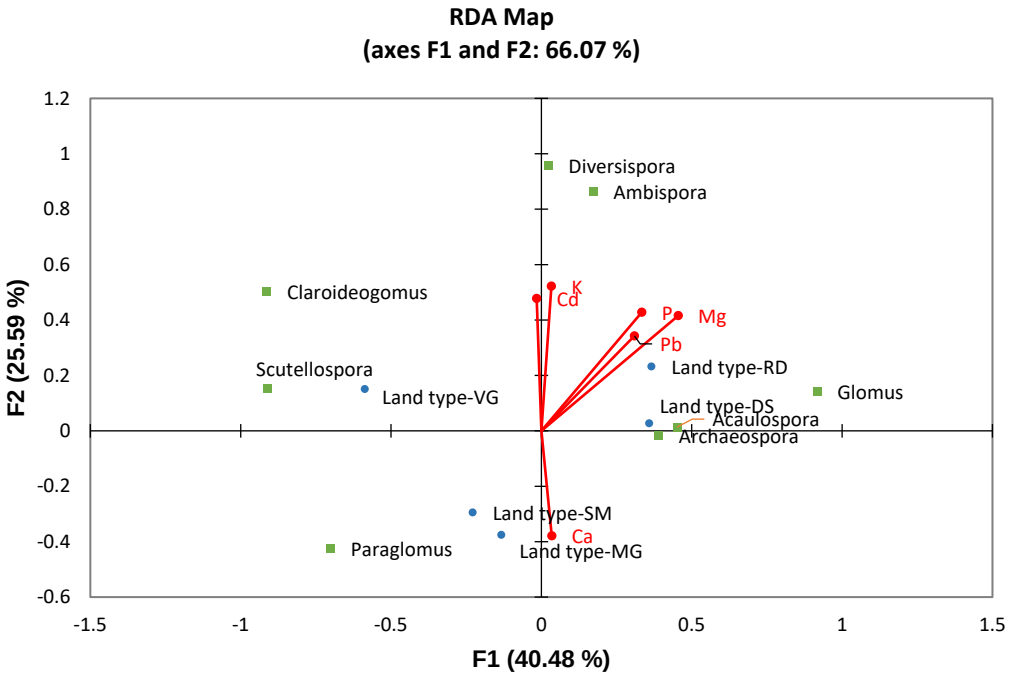

Figure 20. Distance-based redundancy plots used to interpret the extent of correlation between land types and trace metals with AMF communities. The land types used in this redundancy test are listed as follows: vegetation/shrub land (VG), saltmarsh (SM), mangrove (MG), desert/barren land (DS) and rawdha (RD)

4.5 Effect of Soil Chemical Properties on AMF Abundance

The goodness-of-fit measure or variation between the dependent variable (i.e. AMF species) and explanatory variables (i.e. soil chemical characteristics) was evaluated according to R^2 or determination coefficient computed by linear regression. According to the R^2 values of each regression model in Figure 21, the highest degree of correlation or goodness-of-fit measure was presented by soil salinity (R^2 = 0.116) and EC (R^2 = 0.117). In other words, approximately 12% of the variability of the dependent variable, AMF species abundance, is explained by soil salinity and EC. In the case of soil pH and TC, regression analysis showed that approximately 8% and 7% of the total variation of AMF abundance was attributed by pH (R^2 = 0.080) and TC respectively (R^2 = 0.066). Given the negative linearity shown by TC, pH, salinity and EC, we can conclude that these soil attributes produce a negative effect on AMF abundance. Therefore, increasing pH and salinity will likely reduce the overall abundance of AMF in the soil. On the other hand, regressions by both NO_2^- and NO_3^- showed positive linear relationship with AMF. However, only 2% of the variation in AMF abundance was affected by soil NO_3^- (R^2 = 0.016) despite its positive relationship. Similarly, the linear positive relationship between soil NO_2^- (R^2 = 0.064) and AMF abundance only accounted for 6% of the total variation. From this analysis, we can assume that although increasing NO_2^- and NO_3^- in the soil can positively support AMF communities, the overall effect that these nutrients have on AMF communities is insignificant but rather, it is relative to its concentration within the soil environment.

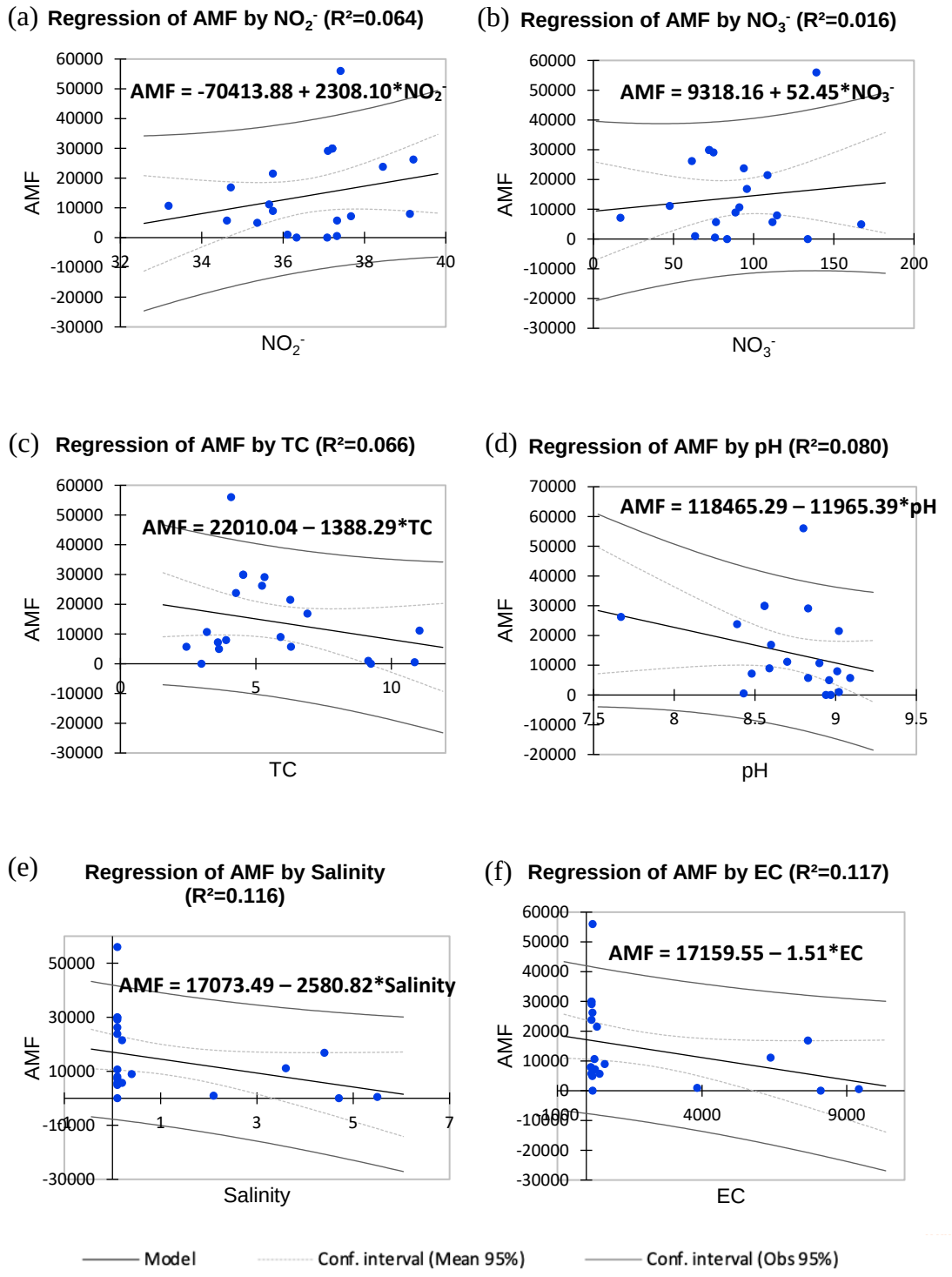

Figure 21. Linear regression analysis of AMF model means indicate positive correlations with (a) NO_2^- (b) NO_3^- and negative correlations with (c) TC (d) pH (e) salinity and (f) EC at 95% confidence interval

The correlation matrix between the chemical characteristics of soil and the abundance of AMF species is shown in Table 22; where results showed that only NO_2^- and NO_3^- had a positive effect on the abundance of AMF species, while the remaining soil properties (TC, pH, salinity, EC and TDS) showed a negative effect on AMF abundance. From the correlation coefficients, it is evident that soil NO_2^- had the most influence on AMF species (0.252), whereas the positive correlation between NO_3^- and AMF was approximately half (0.127) of the influence presented by soil NO_2^-(Table 22). Despite that, increasing NO_2^- and NO_3^- content in the soil environment could potentially increase AMF species. However, increasing TDS, salinity, EC or pH may reduce AMF abundance within the soil environment.

Table 22. Correlation matrix used to assess the relationship between each soil variable and AMF species abundance

	NO_3^-	pH	EC	Salinity	TDS	TC	AMF
NO_2^-	-0.081	-0.491	-0.129	-0.123	-0.136	0.022	0.252
NO_3^-		0.405	-0.055	-0.047	-0.053	-0.148	0.127
pH			-0.055	-0.058	-0.051	-0.100	-0.283
EC				1.000	1.000	0.842	-0.343
Salinity					0.999	0.839	-0.341
TDS						0.843	-0.344
TC							-0.256

The percent variability of the dependent component (abundance of soil AMF species) affected by each trace metal was assessed according to the R^2 or determination coefficient values (Figure 22). About 48% of the variability in AMF abundance was attributed to six trace metals: Ca, Mg, K, P, Cd and Pb and 28% was attributed to the chemical properties listed in Table 22, while the remaining differences in AMF abundance were due to other effects not included in this analysis. However, as one would expect, a large extent of AMF variability was attributed to soil K ($R^2 = 0.308$) and P ($R^2 = 0.219$) content as these metals explained approximately 31% and 22% of

AMF abundance respectively at 95% confidence interval. Alternatively, only 11% of the variability in AMF was affected by Ca (R^2 = 0.109), which turned out to be the element that had the least influence on AMF abundance and the only one that negatively influenced AMF (indicated by a downward slope on the plot), while all other metals showed a positive linear relationship with AMF abundance. However, Mg (R^2 = 0.187) explained 19% of AMF variability and thus, was also among the metals that exhibited the lowest effect on AMF. Referring to the computed R^2 values of the trace metals: Cd (R^2 = 0.208) and Pb (R^2 = 0.129), Cd accounted for about 21% of the differences in AMF abundance, almost as much as soil P, while 13% of these differences was attributed by Pb. From regression analysis, we can conclude that although majority of these metals positively influenced AMF, the percent variability of each metal varied.

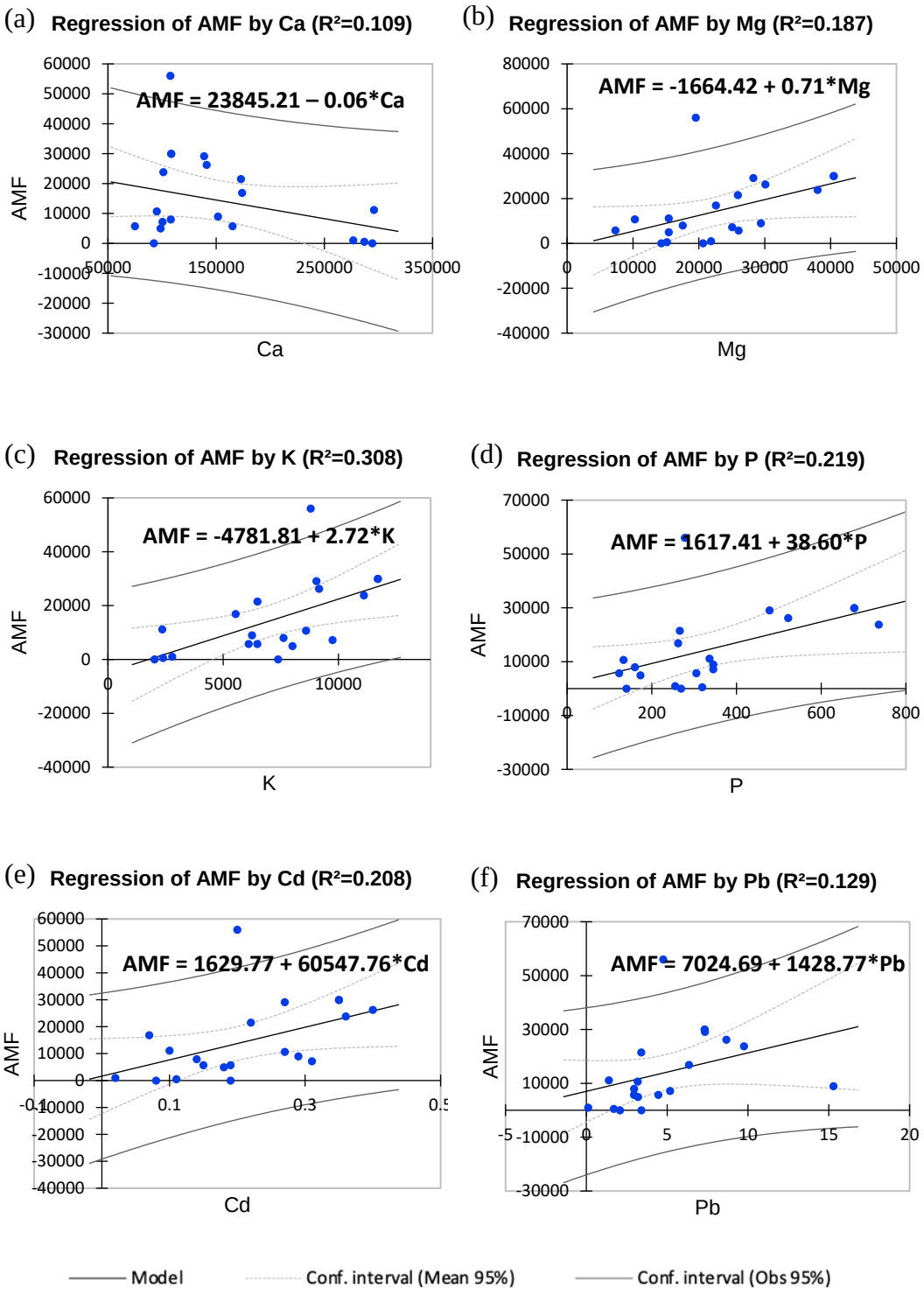

Figure 22. Linear regression analysis of AMF model means indicate negative correlations with (a) Ca and positive correlations with (b) Mg (c) K (d) P (e) Cd and (f) Pb at 95% confidence interval

Results of the correlation matrix (Table 23) showed that Ca had a negative effect on the abundance of AMF species (0.330), while the remaining metals measured in this study produced a positive correlation on AMF abundance, including both heavy metals (i.e. Cd and Pb). However, from the coefficients, the highest positive linearity between the metals and AMF abundance was K and P. Given the necessity of these macronutrients for AMF-plant symbiosis, the positive relationship (indicated by an upward slope on the scatter plots and positive coefficient values) between soil K, P and Mg with AMF was expected. Contrary to initial hypothesis, Cd and Pb produced a positive effect on AMF communities as the positive coefficients (Cd = 0.456 and Pb = 0.360; Table 23) suggest that as Cd and Pb concentrations increase, the total number of AMF species may increase as well.

Table 23. Correlation matrix used to assess the relationship between trace metals and AMF species abundance

	Cd	K	Mg	P	Pb	AMF
Ca	-0.609	-0.857	-0.084	-0.021	-0.384	-0.330
Cd		0.838	0.605	0.632	0.694	0.456
K			0.501	0.473	0.517	0.555
Mg				0.888	0.656	0.433
P					0.561	0.468
Pb						0.360

Despite the negative and positive relationship between the analysed trace metals and number of AMF species, the value or importance that these metals have on AMF abundance may only be considered according to the significance (i.e. p-values) of these effects. The significance values of K ($p = 0.014$), P ($p = 0.043$) and Cd ($p = 0.050$) were less than 0.05 (i.e. 95% confidence interval) and therefore, indicated that the data brought by the explanatory variables: K, P and Cd, were statistically significant. In other words, the effect of these metals on AMF abundance are of significant value. On

the other hand, despite the positive influence of Mg and Pb and negative influence of Ca on AMF abundance, the effect of these remaining elements Ca ($p = 0.168$), Mg ($p = 0.064$), and Pb ($p = 0.130$) were not statistically significant since the p-values computed was greater than 0.05 significance level. On the other hand, the effect of pH and salinity on AMF was not statistically significant as one would expect it to be (Table 24). The significance of each chemical variable were as follows: NO_2^- (0.297), NO_3^- (0.605), TC (0.290), pH (0.240), salinity (0.154), EC (0.151) and TDS (0.150); the p-values were greater than 0.05 and therefore, suggest that the effect of these chemical variables on AMF species abundance was not statistically significant at 95% confidence interval. This analysis implied that soil chemistry was not the predominant factor influencing AMF abundance but was rather, attributed to a combination of environmental factors including land-type and vegetation density.

Table 24. Multiple linear regression was used to evaluate the statistical significance of each chemical variable on AMF while all other variables were held constant. Bold values are statistically significant at 95% confidence interval (p-value < 0.05)

Model	Standardized correlation	Sig.
NO_2^-	0.252	0.297
NO_3^-	0.127	0.605
TC	-0.256	0.290
pH	-0.283	0.240
Salinity	-0.341	0.154
EC	-0.343	0.151
TDS	-0.344	0.150
Ca	-0.330	0.168
Mg	0.433	0.064
K	0.555	**0.014**
P	0.468	**0.043**
Cd	0.456	**0.050**
Pb	0.360	0.130

CHAPTER 5: DISCUSSION

5.1 Influence of Land Type on Soil Chemistry

The sites included in this study varied in environmental conditions, however, much of Qatar's climate is generally distinguished as hyper-arid. In natural conditions, much of Qatar's topography is characterized by a dense layer of loose soil, minimal vegetation coverage and large quantities of coarse gravel. There was a small degree of variation in the chemical parameters between different sampled locations, however, the highest variation was evidently found in areas of mangroves and saltmarshes. These study sites exhibited highly saline soil conditions that are largely dominated by halophyte plants. There were no significant differences in soil TN and NO_2^- concentrations among each land type (that is, mangrove, saltmarsh, sabkha, rawdha and coastal area) as the concentrations measured fell within a small range; due to low concentrations of TN (i.e. less than 0.1%), TN measurements were rendered as negligible. There was, however, significant variation in the concentration of Ca among the land types. High Ca concentrations were evident in highly saline soils such as those in the mangroves and rawdha. *Avicennia marina* plants prevailed in AlKhor mangroves while *Prosopis juliflora* plants prevailed in AlKharrara, an area that presented regions of soil depressions and grass patches. Keeran et al. (2018) described *Prosopis juliflora* as a hyperaccumulator; a plant that has the ability to actively uptake nutrients and heavy metals from the soil environment. Given the phreatophytic nature of *P. juliflora* plant species, its deep roots have allowed it to flourish in hyper-arid environments, particularly in regions where soils lack minerals and organic matter. Present *P. juliflora* plants, along with other phreatophytic plants in AlKharrara sampling site could account for the increase in Ca content in AlKharrara compared to other study sites. Among the sampled locations, sites with higher coverage of bare land exhibited lower soil nutrient

compared to those with more vegetation cover. In this regard, we can conclude that the soil chemical components are largely variable and are generally based on the type of habitat and dominant plant species in the area. However, as in most drylands, existing plant and vegetation types are well-adapted to the adverse environmental conditions and lack of soil nutrients (Delgado-Baquerizo et al., 2015), which is also seen in this study as species of desert plants have shown to be the most prevalent in Qatar's soil, given the low levels of soil TC, TN and trace elements across most sites.

Increasing vegetation coverage in some study sites led to an accumulation of plant litter and root exudates, which have been shown to increase the TN, OC, nitrate and fungal abundance in the soil (Zhang et al., 2017). Contrary to Zhang et al.'s study (2017), fungal and chemical data derived from this study indicated little association between TN, NO_3^- and fungal diversity with vegetation coverage. Moreover, the quantity of Ca and Mg fluctuated between sites and were found to be higher in soils from undisturbed study sites but not in all locations than compared to sampled areas that were in close proximity to the city, urban structures or roads. The highest salinities were measured in sites 2, 3 and 10; where sites 2 and 3 were undisturbed areas of mangroves and saltmarshes respectively, while the sampled soil from location 10 was collected from the biology field at Qatar University, thereby suggesting a relationship between the measured salinities and land type (i.e. lowland and coastal areas). The results attained indicated that similar land types share relatively similar chemical characteristics of soil and thus, follows a comparable pattern of data reported in similar studies (Oehl et al., 2010; Siles et al., 2015; Tian et al., 2017).

5.2 Effect of Soil Chemical Properties on AMF Communities

76% of the variation in AMF communities was explained by the chemical factors included in this study, with soil K (31%) and P (22%) having the largest

explanatory power. It was initially hypothesized that an increase in soil salinity would reduce the abundance of AMF communities. According to (Becerra et al., 2014), highly-saline soil conditions have adverse effects on root colonization which in turn, prevents successful plant-AMF associations from occurring. However, the effect of salinity alone on AMF abundance (Figure 21) was not statistically significant since its p-value (0.154) was greater than 0.05 significance level. The negative correlation of salinity alone on AMF only attributed to 12% of the total variation of AMF communities (Figure 21). It is likely that present AMF in Qatar has been exposed to high salinity levels through their evolutionary history in the region. This prolonged exposure favoured AMF with a natural resistance to salinity through natural selection. From the data obtained, we can conclude high soil salinity, particularly in lowland areas and regions that were within close proximity to Qatar's coasts (e.g. AlKhor, AlThakira, Dukhan and AlKiranah), created extreme soil conditions that restricted fungi to flourish. This in turn, reduced the overall abundance and diversity of fungal species present in these locations and supported the colonization of halotolerant fungi including *Glomus* species.

Initial hypothesis also stated that AMF abundance and diversity was positively associated with increasing quantities of macronutrients (that is, TC, Ca, Mg, P, K, NO_2^- and NO_3^-). While this assumption was true for Mg, K, P, NO_2^- and NO_3^-, results in this study showed that increasing TC and Ca reduced AMF abundance. Results of similar ecological studies on soil fungi (Lauber et al., 2008; Tian et al., 2017) demonstrated that fungal communities were mainly driven by soil P content. In other words, these studies suggested that the diversity and abundance of fungal communities are often correlated with the concentration of extractable soil P; these findings are consistent with the results in this study as the effect of K and P on AMF abundance were statistically

significant. The importance of soil chemistry and macronutrients to microbial communities have been demonstrated in studies involving the use of high-throughput sequencing techniques (Mendoza et al., 2011; Wang et al., 2017; Xue et al., 2018). Higher concentrations of macronutrients (i.e. NO_2^- and NO_3^-) were evident in areas with more vegetation coverage, such as that shown in AlThakira saltmarsh and AlKharsaah. In terms of the trace metals, we also found that locations with more vegetation coverage and plants including QU fields presented higher concentrations of Mg, K and P compared to locations of barren land. Study sites with vast spaces of barren land showed lower concentrations of NO_3^-, NO_2^- and trace metals. The results in this study indicated that these land types, that is, those that presented natural vegetation and grass coverage, positively influenced soil nutrient conditions.

As demonstrated by previous studies (Aldrich-Wolfe, 2007; Tian et al., 2017; Wu et al., 2018), the structure of microbial communities are generally driven by both soil chemical properties and land-type. The significance of soil physicochemical properties in shaping microbial diversity have been established by numerous studies (Lauber et al., 2009; Liu et al., 2014; Rousk et al., 2010; Tian et al., 2017). The general distribution of soil chemical properties may be attributed to variations in environmental factors such as temperature, soil moisture or land type. These soil properties combined, make up the heterogeneity of the environment, which in turn, influences fungal composition in various habitats. Similar relationship between environmental factors and fungal community structure have been shown in other studies (Hossain & Sugiyama, 2011; Vyas & Kumar Gupta, 2014; Xue et al., 2018).

Study sites located in the north-east of Qatar were largely dominated by coastal shores, mangroves and extensive vegetation lands (i.e. AlThakira and AlKhor). Given its close proximity to Qatar's coasts and low topographic nature, these sites exhibited

soils with higher pH, salinity, EC and TDS. Accordingly, the distribution and abundance of belowground AMF communities were also evidently lower in these study areas, likely due to the extreme saline conditions and elevated pH levels of the soils. The pattern of fungal diversity around Qatar is therefore, dependent on the surrounding environment and land type, especially in regions of undisturbed soils. These findings were similar to that presented by Tian et al. (2017) and Xue et al. (2018), thus highlighting the importance of soil type and chemical components to fungal communities. The highest pH was presented in coastal areas, where sites were mostly barren with fewer vegetation compared to other sites. However, most sites studied indicated relatively high pH levels, likely as a result of Qatar's arid climate. Arid lands like Qatar are characterized by low rainfall and high atmospheric temperatures, thereby contributing to high rates of evapotranspiration and little leaching; these factors combined causes ions including carbonates and bicarbonates of calcium and magnesium to accumulate in the soil, especially in areas of low altitudes due to its close vicinity with the seawater. According to Rengasamy (2009), highly alkaline soils are strongly associated with high toxic quantities of iron, carbonate and bicarbonate, nutrient deficiency and elevated sodium levels. The accumulation of exchangeable sodium ions found in arid soils can potentially reduce the physical stability and overall fertility of the soil, thus resulting in an inadequate habitat for AMF root colonization. This is consistent with the results presented in this study as regression analysis indicated that increasing soil pH negatively influences AMF communities by reducing the number of AMF species.

5.3 Potential Sources of Heavy Metals (Cd and Pb) in different Land Types

The highest Pb and Cd concentrations in this study was evidently reported in soils collected from QU field and AlNu'man respectively. Given the petroleum-driven

aspect of Qatar's economy, Pb and Cd contaminated soils in these built-up lands could potentially be a result of industrial runoff, domestic sewage and atmospheric deposits from fuel combustion. In this study, the highest heavy metal contamination was mainly found in built-up areas and cultivated spots close to urban development vegetation land. Moreover, heavy metal accumulation in low-lying flatlands (AlNu'man and AlGhuwayriyah) would have likely been attributed to human-induced activities through surface run-off and atmospheric particles. Trace metals such as Cd and Pb are major constituents of crude oil (Osuji & Onojake, 2004). Therefore, spillages and effluents from petroleum industries located in the north and west of Qatar (Qatar Petroleum offshore petroleum field in the north and Dukhan onshore industrial plant in the west) could strongly influence the Cd and Pb concentrations seen in these areas. However, heavy metal contamination evident in sampled areas close to urbanization could likely be affiliated with wastewater effluents, use of chemical fertilizers and pharmaceutical waste, all of which have reported to be common sources of Cd and Pb (X. Yan et al., 2018). On the other hand, lower Cd and Pb concentrations evident in AlKharrara and AlRayyan may be attributed to long-term weathering of pavements during the summer and exhaust emissions from heavy traffic, thereby leading to atmospheric deposition of Cd and Pb particulate matter, both of which have been reported as main sources of heavy metal contaminants near roadsides (Fakayode & Olu-Owolabi, 2003).

Heavy metal pollution is generally introduced into the soil environment through anthropogenic sources. At high concentrations, Cd and Pb could negatively influence plant productivity within the soil biota (Yan et al., 2018) and in turn, could potentially impact AMF assemblages as mycorrhizal associations are not adequately supported. Combined with Qatar's rapid urbanization, the nation's increasing demand for food resources and industrial material has transpired the country into one that is heavily

dependent on oil-based resources and fertilizer-induced agricultural practices. Treated wastewater effluents are one of the primary sources of water for agricultural purposes and biofuel production. Although a large extent of wastewater pathogens and sludge have been filtered by waste management systems prior to agricultural use, a small portion of heavy metals including Cd and Pb often persist (Farid, 2015). Additionally, petrochemical industries, coal combustion, vehicle emissions, weathering of pavement and building surfaces and atmospheric deposits all combine to increase heavy metal contamination in soil environments. Several studies (Pan et al., 2020; X. Yan et al., 2018) reported that reclamation of coastal wetlands and development of oilfields have lead the concentration of heavy metals (particularly Pb) to levels higher than the average background levels. It was also shown that Cd and Pb content tended to accumulate in residential and urban areas (Antonijević et al., 2012; Imperato et al., 2003) and therefore, supports the assumption that accumulation of these heavy metals could likely have originated from anthropogenic sources.

5.4 Effect of Cd and Pb Fractions on AMF Distribution

According to Lauber et al. (2008) and Pan et al. (2020), the distribution and abundance of soil fungal communities are largely influenced by heavy metals in addition to other well-known AMF drivers (that is, pH, salinity, K, P and NO_2^-). Another study reported that heavy metals including, particularly Cd, not only altered the soil chemistry, but it also posed detrimental effects on microbial communities which lead to losses in diversity and soil fertility (Kasemodel et al., 2019). Even in small quantities, Lin et al.'s study (2019) showed negative correlations between available soil Cd and dominant fungal families. Findings in this study showed similar patterns to those reported in Lin et al. (2019) as Pearson correlation analysis indicated that Cd was

strongly correlated with soil K content which in turn, supported *Acaulospora* and *Archaeospora* AMF species more than the dominant *Glomus* species.

However, in terms of AMF abundance, Cd and Pb indicated a positive linear relationship with the AMF species identified in this study and thus, contradicted the results reported in previous studies (Fakayode & Olu-Owolabi, 2003; Kasemodel et al., 2019; Song et al., 2018). The relationship between the heavy metals and AMF abundance also opposes our initial hypothesis since increasing heavy metal content in the soil does not reduce AMF abundance, as one would expect. Although heavy metals have often been associated with a direct negative effect on soil microbial communities (Pan et al., 2020; Song et al., 2018); the results achieved in this study suggest otherwise, as regression analysis indicated that increasing Cd and Pb concentrations tended to increase AMF abundance. Moreover, the positive effect of Cd on AMF abundance was statistically significant. These differences may be due to the possibility that more resistant fungal communities such as *Glomeraceae* fungi are present within Qatar's soil environment, given the extreme environmental conditions of Qatar's habitat. Moreover, species from *Glomeraceae* family have shown to be more resilient in nature compared to other fungal phyla (Oyediran et al., 2018; Zhao et al., 2017). Alternatively, negative correlations between *Blastocladiomycota* phyla and heavy metals (mainly Pb, Cd and Zn) in another study (Pan et al., 2020) were only evident in the water-extractable fractions of these metals, therefore suggesting that the water-extractable portions of Pb and Cd may exert greater influence on soil fungal assemblages since these fractions demonstrate higher mobility and are therefore, likely to be more bioavailable within the soil (Tripathy et al., 2014).

It was initially hypothesized that increasing Cd and Pb in the soil would hinder mycorrhizal associations and ultimately, reduce AMF diversity and abundance.

According to Lin et al. (2019), soil fungi are generally sensitive to environmental changes like pH, heavy metals and soil nutrients and thus, are considered to be useful bioindicators of soil contamination. While other studies have reported several adverse effects of Pb and Cd-contaminated soil on fungi (Baldrian, 2003; Dietterich et al., 2017), this study suggested otherwise as RDA plots showed that soil P and K were the main drivers of AMF distribution, while present Pb and Cd demonstrated less effect on the distribution of AMF families. Furthermore, field studies focusing on the effect of heavy metals on soil AMF distributions have shown inconsistent results; elevated soil metals reduced AMF colonization in some habitat systems (Khan, 2001) but increased AMF diversity in others (Vogel-Mikuš et al., 2006). It is also likely that fungal assemblages tolerate heavy metal-induced stress by limiting uptake or sequestering toxic metals in intracellular compartments that reduce the overall toxicity of soil Pb and Cd (Dietterich et al., 2017).

AMF tolerance to heavy metals have been reported in previous studies (Kõljalg et al., 2013; Weissenhorn et al., 1993); the highest reported tolerant strain of AMF was *Glomus* species. Weissenhorn et al.'s study (1993) showed that Cd had no effect on *Glomus mosseae* spore germination, despite elevated Cd concentrations in soil extracts. The extent of tolerance presented by *Glomus* fungi were also expressed at the hyphal level as Cd (0.38 mg/kg) indicated little to no effect on hyphal development; Cd concentrations measured in this study were well below the quantities reported by (Weissenhorn et al., 1993) and thus, could imply that AMF, particularly *Glomus sp.*, are well-adapted to withstand Cd and Pb contaminated soils. Jiang et al. (2016) reported that *Glomus* inoculation enhanced plant productivity and supported hyphal extensions by improving P absorption in plant roots and shoots which in turn, allowed existing plants to successfully support AMF colonization within their root systems. Moreover,

results of (Bissonnette et al., 2010) showed that although Cd and Pb concentrations in plant shoots decreased, these heavy metals significantly increased in the roots, likely as a result of Cd and Pb mycorrhizoremediation by AMF. The degree of heavy metal tolerance may differ between AMF genera (Morton, 1986). Hildebrandt et al., (2006) reported that the main cause of heavy metal toxicity was oxidative stress. Results from other studies (Moons, 2003; Smith et al., 2004) reported an up-regulation of a glutathione *S*-transferase (GST) gene (4b07), induced by Cd and Pb exposure, that provided a protective mechanism against oxidative stress and consequently, alleviated Cd or Pb toxicity in the intracellular mycelium of AMF. Given the undeterred increase in AMF abundance in Cd and Pb contaminated soils, the data obtained could potentially demonstrate the expression of gene-encoding proteins as a response to heavy metal stress. However, given the lack of data on metal toxicity and AMF mechanisms in Qatar, further research should focus on addressing the response mechanisms of AMF species to the water extractable Pb and Cd fractions in soil.

5.5 AMF Diversity

The AMF taxa identified in this study primarily belonged to the phylum: Glomeromycota and order: Glomerales, Diversisporales, Paraglomerales and Archaeosporales. Although *Glomus*, *Acaulospora, Ambispora, Archeospora, Claroideoglomus, Diversispora, Scutellospora* and *Paraglomus* species were identified in this study, species from the *Glomus* genus was presented as the major fungal group in most of the locations and in all the habitat-types (sabkha, mangrove, saltmarsh, vegetation land). Representatives of *Glomus* genus therefore, served as the indicator species for each site. These results are supported by similar findings in previously published studies (Oehl et al., 2010; Zhao et al., 2017), confirming *Glomus* fungi as the most common genus within the AMF assemblages in Qatar. Despite the diversity of

AMF reported in this study, Tian et al. (2017) reported that much of AMF species are more inclined to inhabit cooler arid environments due to their evolutionary histories, as opposed to hotter dry habitats.

Studies reported by Kasel et al. (2008) and Leckie et al. (2004) showed that land-type played a major role in modulating the structure of fungal communities. However, Lauber et al. (2008) determined that soil chemical parameters had a stronger influence on soil fungal communities than did land-type. In this study, redundancy analysis indicated that the concentration of available P was the predominant controlling factor, and thus, were consistent with the results demonstrated by Lauber et al. (2008). Sites with similar concentrations of K also tended to cluster together. This clustering could likely be related to corresponding microclimates within the soil environment for each habitat type (e.g. coastal shore, mangrove area or sabkha), or influenced by the role of fungi itself within its niche. The distribution of AMF was also related to TC, NO_2^- and NO_3^- to a certain extent; however, AMF diversity and abundance was more strongly correlated with soil pH and salinity. In contrast, Lauber et al. (2008) found that distribution of soil fungal communities were related to the P content as his study indicated higher abundance of *Ascomycota* compared to *Basidiomycota* in P-rich soils. On the other hand, Tian et al. (2017) reported higher content of *Basidiomycota* in P-rich soils. Discrepancy between the two studies may be influenced by the range of soil phosphorous content; concentration of soil P was measured to be in the range 1.8-17 mg/kg in Lauber et al.'s study (2008) and 1.65-3.31 mg/kg in Tian et al.'s study (2017).

Alternatively, the presence of *Glomus* species is essentially responsible for the decomposition of plant litter due to its saprotrophic nature. *Glomus* species can therefore, alter soil chemical properties in areas with abundant plant litter as the likelihood of plant litter decomposition increases (Frederikke et al., 2010; Kuramae et

al., 2013; Tian et al., 2017). Among the sampled locations in this study, higher quantities of plant litter were present in sites with more vegetation coverage and trees compared to sites with bare land or little to no plants, thereby resulting in slight differences in the soil chemical properties. However, Tian et al. (2017) reported that *Ascomycota* species have a higher likelihood of thriving in areas of barren land as opposed to other fungal species. The high abundance of *Glomus* species recorded in this study contradict the results presented by Tian et al. (2017), who explained that approximately 46% of *Ascomycota* form lichens, a mutualistic association between composite plant filaments and fungi, which supports its successful growth in arid desert habitats. Among the locations presented in this study, sites with poor vegetation cover or vast spaces of barren land are generally more reflective when in direct contact with sunlight and as a result, exhibit lower fungal abundance and smaller fungal communities. Combined with continuous drought and extremely arid conditions in Qatar, rhizosphere-fungal associations can remain dormant for long periods of time, thereby allowing them to tolerate the dry and hot environmental conditions (Tian et al., 2017; Xue et al., 2018). According to the AMF families presented in this study, we can infer that different land-types affect the distribution of AMF in terms of those that are most adapted to the extreme saline and dry conditions of Qatar's habitat, that is, primarily *Glomus* communities. This could justify the difference in results found in areas with dense vegetation compared to bare land as the quality of soil is altered with increasing vegetation and consequently, affecting the type of soil fungi present.

The ability of desert plants and vegetation in arid regions to secrete large amounts of sugars and amino acids for AMF growth is one of the unique features that allows AMF communities to thrive in highly arid climatic environments (Oyediran et al., 2018). Coupled with the relatively low P content, particularly in areas of scarce

vegetation coverage, the lack of plants in these study areas may have contributed to lower AMF diversity and population. However, in some sites, soils that comprise of higher fractions of loose sand like those collected from sabkha regions may have allowed increased aeration for humus or organic matter decomposition and nutrient penetration (Torrecillas et al., 2014), thereby enhancing fungal propagation and growth and as a consequence, increased AMF population.

5.6 AMF Abundance between Sites

Higher diversity of AMF families recorded in soil samples collected from QU biology fields may be attributed to human intervention as these areas indicate more vegetation coverage as a consequence of runoff, irrigation or the use of fertilizers in nearby areas to increase soil fertility and support plant growth. Additionally, adequate water supply has been reported to directly impact the composition and distribution of AMF communities by enhancing the biological nature of the soil and ecological niche of AMF communities (Krasensky & Jonak, 2012), which supports the results achieved in this study as sampled regions that demonstrated a certain extent of water availability such as the QU fields indicated comparatively higher AMF diversity. In other words, changes in the distribution of AMF communities may be a result of the water content due to its importance in reproductivity and metabolic processes (Zhao et al., 2017). On the other hand, despite water availability, lower AMF diversity was recorded in the mangrove and saltmarsh regions, likely due to the salt and temperature-induced stress conditions in these regions. Combined with high atmospheric temperatures and low rainfall, study sites indicated by high salinities, pH and Ca content generally presented lower diversity of AMF communities. In another study, Bueno et al. (2017) reported that increasing aridity hindered root colonization and subsequent mycorrhizal associations. Bueno et al. (2017) also observed that the amount of available water in

extreme dry conditions tended to recede in smaller pores of soil aggregations which reduced contact between penetrable pores and water layers within the soil. In van der Heijden et al.'s study (2015), it was further reported that low concentrations of soil nutrients restricted root colonization and AMF production.

Results showed that the highest Shannon index values pertaining to the study sites were recorded from QU field, AlKharsaah and AlRayyan samples. This outcome may have been a result of increased soil nutrient availability and plant host as the vegetation coverage and plant occurrence was evidently higher in these areas compared to other sampled sites. It is also generally known that water availability from cultivation practices or natural runoff and soil nutrient content are regarded as useful indicators of AMF diversity (Aroca et al., 2007). On the other hand, the lowest Shannon diversity indices were recorded from the AlThakira saltmarsh, Khawzan and Kiranah. These regions displayed the lowest diversity of AMF, likely due to the high salinity and pH and relatively low NO_3^- content. Adding to the region's naturally arid climate, these factors combined create a significantly harsh environment that renders the habitat as unfavorable for the growth of most AMF species.

The high number of *Glomus* species recorded from most of the sites in this study is consistent with the results shown in previous findings (Bueno et al., 2017; Gerz et al., 2016); confirming that *Glomus* species are generally the predominant fungal genus in many AMF communities. This could potentially be attributed to its unique ability to tolerate both alkaline and acidic soil conditions and consequently, sustain its growth in extreme soil environments. Additionally, symbiotic interactions between *Glomus* species and existing plants to co-adapt and tolerate unfavorable environmental challenges could potentially be another reason for its apparent abundance in many AMF assemblages (Soudzilovskaia et al., 2015). Another study revealed that *Glomus* species

produce larger quantities of spores and hypha filaments compared to fungal species of other genera; these resulting spores and hypha colonize to create extensive structures that branch onto plant roots (Öpik & Davison, 2016) which increases the likelihood of *Glomus* species to survive in more extreme environments. Bennett et al. (2017) reported that extensive intermingling of *Glomus* mycelium filaments increases the efficiency of soil nutrient and water exploitation and carbon translocation, therefore improving nutrient uptake within fungal-root-plant associations in regions of extreme aridity (Kamalvanshi et al., 2012; Oyediran et al., 2018). Another study also reported that drought-adapted *Glomus* species are critical biological components in the modulation of gas exchange between the soil environment and plants (Querejeta et al., 2006) which consequently, facilitates long-term development of *Glomus*-associated host plants in arid ecosystems. In further research, Querejeta et al. (2006) also theorized that native *Glomus* species in arid soil environments improved the water status and transpiration efficacy of host plants. The ability of *Glomus* species to adapt and thrive in arid soils therefore, plays a vital role in the growth of its host plants and consequently, colonization of other fungal families.

The positive correlation between AMF abundance with soil NO_2^- and NO_3^- could likely be a result of continuous nutrient supply to plant shoots and roots (Vyas & Kumar Gupta, 2014), which in turn, enhances mycorrhizal associations in the root systems (Cheng et al., 2012; Oyediran et al., 2018). Other AMF-related studies that also observed higher occurrence of mycorrhizal associations explained that the prevalence of AMF may be attributed to nutrient availability, specifically the composition of OC, N, P and K, among other chemical factors (Bagyaraj & Revanna, 2017; Gerz et al., 2018). The relatively low AMF diversity observed in several sites could potentially be a result of low TN and P availability, especially in areas of scarce vegetation, which

further supports the interdependent relationship between AMF community structure and available nutrients. These findings agree with those mentioned by (Bhat et al., 2014; Timmer & Leyden, 1980), but contradicts the results reported by (Khanam et al., 2006) who concluded a negative correlation between AMF population and K content. Khanam et al. (2006) reported that although soil K remains to be an essential component in AMF diversity and abundance, it may not be the predominant component since AMF communities may also be driven by a combination of other environmental factors. In addition to soil K, results reported in Zhao et al.'s study (2017) revealed that soil P also contributed a significant impact on AMF community structure as available P stimulated hyphal development and fungal spore germination (Miranda & Harris, 1994). On the other hand, the effect of soil K on AMF communities is more indirect as K increases the expression of two potassium-channel genes (LbKT1 and LbSKOR) in plant roots under drought and arid conditions (Zhang et al., 2017), which in turn, supports AMF colonization. This further confirms the effect of different soil components and environmental conditions on the composition and abundance of AMF communities.

CHAPTER 6: CONCLUSION AND FUTURE PERSPECTIVES

This study first delineated the composition and species diversity of AMF communities around Qatar. Fungal species of the phylum *Glomeraceae* were the most dominant across all soil types sampled in this study. The results achieved suggested that soil chemical composition was the predominant factor influencing AMF assemblages as the chemical properties investigated in this study accounted for 76% of AMF variability. Additionally, this study also indicated slight variations in the composition and occurrence of AMF species among different habitat types as the occurrence and diversity of AMF families were relatively higher in areas that presented more vegetation coverage. These findings present new information on the characteristics of soil community structure and chemical drivers of fungal communities in hyper-arid desert environments such as Qatar; while also highlighting the potential significance of different habitat types on AMF communities.

It was initially hypothesized that an increase in soil salinity and pH will reduce the total abundance and diversity of AMF species. Although regression analysis indicated a negative correlation between salinity, pH and AMF; this assumption was proven not to be true as the significance of these soil components (pH and salinity) on AMF abundance was not statistically significant. It is likely that present AMF in Qatar has been exposed to high salinity levels through their evolutionary history in the region. This prolonged exposure favoured AMF with a natural resistance to salinity through natural selection. From this, we may conclude that high soil salinity, particularly in lowland areas and regions that were within close proximity to Qatar's coasts (e.g. AlKhor, AlThakira, Dukhan and AlKiranah), created extreme soil conditions that restricted fungi to flourish. This in turn, reduced the overall abundance and diversity of fungal species present in these locations and supported the colonization of halotolerant

fungi, particularly *Glomeraceae* fungi. Initial hypothesis also presumed that increasing macronutrients in the soil (i.e. TC, Ca, Mg, K, P, NO_2^- and NO_3^-) would strengthen AMF colonization and ultimately, increase AMF abundance and diversity. While this assumption was true for Mg, K, P, NO_2^- and NO_3^-, results in this study showed that increasing TC and Ca reduced AMF abundance. Alternatively, our third hypothesis pertaining the heavy metals, Cd and Pb, was also proven to be incorrect as regression analysis indicated a positive relationship between Cd and Pb and AMF abundance. However, the negative effects of TC, Ca, Mg and Pb on AMF were not statistically significant at 95% confidence interval.

The present study concludes that (1) *Glomeraceae* is the dominant family among the eight AMF phyla identified in this study. Indicator species analysis also presented *Glomus* species as the indicator species in 15 locations. (2) Variation in AMF population and abundance between locations were mainly attributed to the availability of macronutrients, primarily soil P and K. These chemical components combined make up the driving factors that shape the distribution of AMF soil communities across Qatar's arid regions. (3) Although the linear relation between soil Pb and AMF was not significant, AMF abundance was however, positively influenced by both Cd and Pb and was shown to be significantly influenced by Cd. Despite that, this study lacked sufficient data addressing the metabolic response of AMF in Pb and Cd-contaminated soils. Further research should therefore, focus on the metabolic pathways of AMF assemblages associated with heavy metal toxicity. It is also worthwhile that future microbial ecological studies investigate AMF-plant-metal interactions in hyper-arid habitats, particularly in the Middle East region, due to the lack of sufficient data pertaining these interactions.

The results achieved in this study provide extensive knowledge on the chemical drivers and diversity of AMF communities in an ecologically-stressed environment. This study presents promising findings that forms a key foundation to better understand the important functions that AMF communities play within the arid regions of Qatar's habitat. These findings provide baseline information on the role of AMF in enhancing the distribution of ecological resources and increasing the tolerance of plants and vegetation to certain prevailing environmental stressors in Qatar's extreme agro-environment. Such findings could potentially provide researchers with the tools to delve deeper into the functional profiles of AMF communities and its symbiotic role with various vegetation types, whether natural or introduced, in areas that display edaphoclimatic conditions, such as that in Qatar.

REFERENCES

Abulfatih, H. A., Abdel Bari, E. M., Alsubaey, A., & Ibrahim, Y. M. (2002). Halophytes and soil salinity in Qatar. *Qatar University Science Journal*, *22*, 119–135.

Al-Tamie, M. S. S. (2014). Effect of Salinity on the Fungal Occurance in Al-Shega Area at Al-Qassim, Saudi Arabia. In *Research Journal of Microbiology* (Vol. 9, Issue 6, pp. 287–295). https://doi.org/10.3923/jm.2014.287.295

Aldrich-Wolfe, L. (2007). Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. *Ecology*, *88*(3), 559–566. https://doi.org/10.1890/05-1177

Aliasgharzadeh, N., Saleh Rastin, N., Towfighi, H., & Alizadeh, A. (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza*, *11*(3), 119–122. https://doi.org/10.1007/s005720100113

Anjan Kumar Prusty, B., Chandra, R., & Azeez, P. A. (2009). Distribution of carbon, nitrogen, phosphorus, and sulfur in the soil in a multiple habitat system in India. *Australian Journal of Soil Research*, *47*(2), 177–189. https://doi.org/10.1071/SR08087

Antonijević, M. M., Dimitrijević, M. D., Milić, S. M., & Nujkić, M. M. (2012). Metal concentrations in the soils and native plants surrounding the old flotation tailings pond of the Copper Mining and Smelting Complex Bor (Serbia). *Journal of Environmental Monitoring*, *14*(3), 866–877. https://doi.org/10.1039/c2em10803h

Aroca, R., Porcel, R., & Ruiz-Lozano, J. M. (2007). How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in <i>Phaseolus vulgaris<i> under drought, cold or salinity stresses? *New*

Phytologist, *173*(4), 808–816. https://doi.org/10.1111/j.1469-8137.2006.01961.x

Asemaninejad, A., Weerasuriya, N., Gloor, G. B., Lindo, Z., & Thorn, G. (2016). New primers for discovering fungal diversity using nuclear large ribosomal DNA. *PLoS ONE*, *11*(7), 1–15. https://doi.org/10.1371/journal.pone.0159043

Babikir, A. A. A. (1990). The Vegetation of the State of Qatar as Related to Landform and Soil. *Journal of the Documentation and Humanities Research Center*, 462–478. https://doi.org/http://hdl.handle.net/10576/8409

Bagyaraj, D. J., & Revanna, A. (2017). *Can Mycorrhizal Fungi Influence Plant Diversity and Production in an Ecosystem?* https://www.researchgate.net/publication/305688897_Can_Mycorrhizal_Fungi_Influence_Plant_Diversity_and_Production_in_an_Ecosystem

Baldrian, P. (2003). Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology*, *32*(1), 78–91. https://doi.org/10.1016/S0141-0229(02)00245-4

Bansal, O. (2018). The Influence of Potentially Toxic Elements on Soil Biological and Chemical Properties. *Intech*, 13. https://doi.org/http://dx.doi.org/10.5772/57353

Baumann, K., & Marschner, P. (2013). Effects of salinity on microbial tolerance to drying and rewetting. *Biogeochemistry*, *112*(1–3), 71–80. https://doi.org/10.1007/s10533-011-9672-1

Becerra, A., Bartoloni, N., Cofré, N., Soteras, F., & Cabello, M. (2014). *Arbuscular mycorrhizal fungi in saline soils: Vertical distribution at different soil depth*. www.sbmicrobiologia.org.br

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: An in silico approach reveals potential PCR biases. *BMC Microbiology*, *10*, 1–9.

https://doi.org/10.1186/1471-2180-10-189

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321). https://doi.org/10.1126/science.aai8212

Benny, G. L., Humber, R. A., & Morton, J. B. (2001). Zygomycota: Zygomycetes. In *Systematics and Evolution* (pp. 113–146). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-10376-0_6

Benschneider, K., & Robinson, R. (1952). New spectrophotometric method for the determination of nitrite in water. *Journal of Marine Research*, *11*, 87–96. https://doi.org/10.1007/BF00388430

Bhat, B., Sheikh, M., & Tiwari, A. (2014). *Impact of various edaphic factors on AMF spore population and diversity in Catharanthus roseus at Gwalior*. https://www.researchgate.net/publication/297544503_Impact_of_various_edaphic_factors_on_AMF_spore_population_and_diversity_in_Catharanthus_roseus_at_Gwalior

Bissonnette, L., St-Arnaud, M., & Labrecque, M. (2010). Phytoextraction of heavy metals by two Salicaceae clones in symbiosis with arbuscular mycorrhizal fungi during the second year of a field trial. *Plant and Soil*, *332*(1), 55–67. https://doi.org/10.1007/s11104-009-0273-x

Bueno, C. G., Moora, M., Gerz, M., Davison, J., Öpik, M., Pärtel, M., Helm, A., Ronk, A., Kühn, I., & Zobel, M. (2017). Plant mycorrhizal status, but not type, shifts with latitude and elevation in Europe. *Global Ecology and Biogeography*, *26*(6), 690–699. https://doi.org/10.1111/geb.12582

Cheng, L., Booker, F. L., Tu, C., Burkey, K. O., Zhou, L., Shew, H. D., Rufty, T. W.,

& Hu, S. (2012). Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO2. *Science*, *337*(6098), 1084–1087. https://doi.org/10.1126/science.1224304

Datta, P., & Kulkarni, M. (2012). Arbuscular Mycorrhizal Fungal Diversity in Sugarcane Rhizosphere in Relation with Soil Properties. *Notulae Scientia Biologicae*, *4*(1), 66–74. https://doi.org/10.15835/nsb416567

DeBellis, T., Kembel, S. W., & Lessard, J. P. (2019). Shared mycorrhizae but distinct communities of other root-associated microbes on co-occurring native and invasive maples. *PeerJ, 2019*(7). https://doi.org/10.7717/peerj.7295

Delgado-Baquerizo, M., Florentino, A., Gozalo, B., Huber-Sannwald, E., Bran, D., Jankju, M., Barraza-Zepeda, C., Bowker, M. A., Zaady, E., Wang, D., Arredondo, T., Ochoa, V., Miriti, M., Jeffries, T. C., Ulrich, W., Eldridge, D. J., García-Gómez, M., Naseri, K., Gaitán, J., … Gallardo, A. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(51), 201516684. https://doi.org/10.1073/pnas.1516684112

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdugo, M., Campbell, C. D., & Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, *7*, 1–8. https://doi.org/10.1038/ncomms10541

Delgado, A., & Gómez, J. (2016). The Soil. Physical, Chemical and Biological Properties. *Principles of Agronomy for Sustainable Agriculture*, 15–27. https://doi.org/10.1007/978-3-319-46116-8

Dietterich, L. H., Gonneau, C., & Casper, B. B. (2017). *Arbuscular mycorrhizal colonization has little consequence for plant heavy metal uptake in contaminated*

field soils Author (s): Lee H . Dietterich , Cédric Gonneau and Brenda B . Casper Published by : Wiley on behalf of the Ecological Society of America. *27*(6), 1862–1875.

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs*, *67*(3), 345–366. https://doi.org/10.2307/2963459

Dumbrell, A. J., Ashton, P. D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A. H., & Helgason, T. (2011). Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist*, *190*(3), 794–804. https://doi.org/10.1111/j.1469-8137.2010.03636.x

Egger, K. N. (1995). Molecular analysis of EM fungal communities. *Nature*, *215*, 676–676. https://doi.org/10.1038/215676a0

Erich, M. S., & Hoskins, B. R. (2011). Effects of soil drying on soil ph and nutrient extractability. *Communications in Soil Science and Plant Analysis*, *42*(10), 1167–1176. https://doi.org/10.1080/00103624.2011.566961

Fakayode, S. O., & Olu-Owolabi, B. I. (2003). Heavy metal contamination of roadside topsoil in Osogbo, Nigeria: Its relationship to traffic density and proximity to highways. *Environmental Geology*, *44*(2), 150–157. https://doi.org/10.1007/s00254-002-0739-0

Farid, G. (2015). Heavy Metals (Cd, Ni and Pb) Contamination of Soils, Plants and Waters in Madina Town of Faisalabad Metropolitan and Preparation of Gis Based Maps. *Advances in Crop Science and Technology*, *04*(01), 1–7. https://doi.org/10.4172/2329-8863.1000199

Frac, M., Hannula, S. E., Belka, M., & Jędryczka, M. (2018). Fungal biodiversity and their role in soil health. *Frontiers in Microbiology*, *9*(APR), 1–9.

https://doi.org/10.3389/fmicb.2018.00707

Frederikke, M., Bjorbaekmo, M., Carlsen, T., Brysting, A., Vrålstad, T., Høiland, K., Ugland, K. I., Geml, J., Schumacher, T., & Kauserud, H. (2010). *High diversity of root associated fungi in both alpine and arctic Dryas octopetala*. https://doi.org/10.1186/1471-2229-10-244

Gamper, H. A., Walker, C., & Schüßler, A. (2009). Diversispora celata sp. nov: Molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytologist*, *182*(2), 495–506. https://doi.org/10.1111/j.1469-8137.2008.02750.x

Garbeva, P., van Veen, J. A., & van Elsas, J. D. (2004). MICROBIAL DIVERSITY IN SOIL: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology*, *42*(1), 243–270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

Gerz, M., Bueno, C. G., Zobel, M., & Moora, M. (2016). Plant community mycorrhization in temperate forests and grasslands: relations with edaphic properties and plant diversity. *Journal of Vegetation Science*, *27*(1), 89–99. https://doi.org/10.1111/jvs.12338

Gerz, M., Guillermo Bueno, C., Ozinga, W. A., Zobel, M., & Moora, M. (2018). Niche differentiation and expansion of plant species are associated with mycorrhizal symbiosis. *Journal of Ecology*, *106*(1), 254–264. https://doi.org/10.1111/1365-2745.12873

Hannachi, N., Cocco, S., Fornasier, F., Agnelli, A., Brecciaroli, G., Massaccesi, L., Weindorf, D., & Corti, G. (2015). Effects of cultivation on chemical and biochemical properties of dryland soils from southern Tunisia. *Agriculture, Ecosystems and Environment*, *199*, 249–260.

https://doi.org/10.1016/j.agee.2014.09.009

Hedrick, D. B., Peacock, A., Stephen, J. R., Macnaughton, S. J., Brüggemann, J., & White, D. C. (2000). Measuring soil microbial community diversity using polar lipid fatty acid and denaturing gradient gel electrophoresis data. *Journal of Microbiological Methods*, *41*(3), 235–248. https://doi.org/10.1016/S0167-7012(00)00157-3

Hildebrandt, U., Regvar, M., & Bothe, H. (2006). *Arbuscular mycorrhiza and heavy metal tolerance*. https://doi.org/10.1016/j.phytochem.2006.09.023

Hossain, Z., & Sugiyama, S. I. (2011). Geographical structure of soil microbial communities in northern Japan: Effects of distance, land use type and soil properties. *European Journal of Soil Biology*, *47*(2), 88–94. https://doi.org/10.1016/j.ejsobi.2010.11.007

Howard, R. J. (2010). Intraspecific variation in growth of marsh macrophytes in response to salinity and soil type: Implications for wetland restoration. *Estuaries and Coasts*, *33*(1), 127–138. https://doi.org/10.1007/s12237-009-9227-z

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, *82*(3), 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Imperato, M., Adamo, P., Naimo, D., Arienzo, M., Stanzione, D., & Violante, P. (2003). Spatial distribution of heavy metals in urban soils of Naples city (Italy). *Environmental Pollution*, *124*(2), 247–256. https://doi.org/10.1016/S0269-7491(02)00478-5

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., & Barea, J. M. (2003). The

contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. In *Biology and Fertility of Soils* (Vol. 37, Issue 1, pp. 1–16). Springer. https://doi.org/10.1007/s00374-002-0546-5

Jiang, Q. Y., Zhuo, F., Long, S. H., Zhao, H. Di, Yang, D. J., Ye, Z. H., Li, S. S., & Jing, Y. X. (2016). Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of Lonicera japonica grown in Cd-added soils? *Scientific Reports*, *6*(February), 2–10. https://doi.org/10.1038/srep21805

Jing, X., Sanders, N. J., Shi, Y., Chu, H., Classen, A. T., Zhao, K., Chen, L., Shi, Y., Jiang, Y., & He, J. S. (2015). The links between ecosystem multifunctionality and above-and belowground biodiversity are mediated by climate. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms9159

Johansen, R. B., Johnston, P., Mieczkowski, P., Perry, G. L. W., Robeson, M. S., Burns, B. R., & Vilgalys, R. (2016). A native and an invasive dune grass share similar, patchily distributed, root-associated fungal communities. *Fungal Ecology*, *23*, 141–155. https://doi.org/10.1016/j.funeco.2016.08.003

Kamalvanshi, M., Kumar, A., Jha, A., & Dhyani, S. K. (2012). Occurrence of Arbuscular Mycorrhizal Fungi in Rhizosphere of Jatropha curcas L. in Arid and Semi Arid Regions of India. *Indian Journal of Microbiology*, *52*(3), 492–494. https://doi.org/10.1007/s12088-011-0224-0

Kasel, S., Bennett, L. T., & Tibbits, J. (2008). Land use influences soil fungal community composition across central Victoria, south-eastern Australia. *Soil Biology and Biochemistry*, *40*(7), 1724–1732. https://doi.org/10.1016/j.soilbio.2008.02.011

Kasemodel, M. C., Sakamoto, I. K., Varesche, M. B. A., & Rodrigues, V. G. S. (2019). Potentially toxic metal contamination and microbial community analysis in an

abandoned Pb and Zn mining waste deposit. *Science of the Total Environment*, *675*, 367–379. https://doi.org/10.1016/j.scitotenv.2019.04.223

Keeran, N. S., Balasundaram, U., Govindan, G., & Parida, A. K. (2018). Prosopis juliflora: A Potential Plant for Mining of Genes for Genetic Engineering to Enhance Phytoremediation of Metals. A Potential Plant for Mining of Genes for Genetic Engineering to Enhance Phytoremediation of Metals. In *Transgenic Plant Technology for Remediation of Toxic Metals and Metalloids*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814389-6.00018-3

Kelly, R., & Love, N. (2007). Ultraviolet Spectrophotometric Determination of Nitrate: Detecting Nitrification Rates and Inhibition. *Source: Water Environment Research*, *79*(7), 808–812. https://doi.org/10.2175/106143007X156682

Khan, A. G. (2001). Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. *Environment International*, *26*(5–6), 417–423. https://doi.org/10.1016/S0160-4120(01)00022-8

Khanam, D., Mridha, M. A. U., Solaiman, A. R. M., & Hossain, T. (2006). Effect of edaphic factors on root colonization and spore population of arbuscular mycorrhizal fungi. *Bull. Inst. Trop. Agr., Kyushu Univ*, *29*, 97–104.

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K. H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Krasensky, J., & Jonak, C. (2012). Drought, salt, and temperature stress-induced

metabolic rearrangements and regulatory networks. *Journal of Experimental Botany*, *63*(4), 1593–1608. https://doi.org/10.1093/jxb/err460

Kuramae, E. E., Hillekens, R. H. E., de Hollander, M., van der Heijden, M. G. A., van den Berg, M., van Straalen, N. M., & Kowalchuk, G. A. (2013). Structural and functional variation in soil fungal communities associated with litter bags containing maize leaf. *FEMS Microbiology Ecology*, *84*(3), 519–531. https://doi.org/10.1111/1574-6941.12080

Laban, P., Metternicht, G., & Davies, J. (2018). Soil biodiversity and soil organic carbon: keeping drylands alive. In *Soil biodiversity and soil organic carbon: keeping drylands alive*. https://doi.org/10.2305/iucn.ch.2018.03.en

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, *75*(15), 5111–5120. https://doi.org/10.1128/AEM.00335-09

Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, *40*(9), 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Leckie, S. E., Prescott, C. E., Grayston, S. J., Neufeld, J. D., & Mohn, W. W. (2004). Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. *Microbial Ecology*, *48*(1), 29–40. https://doi.org/10.1007/s00248-003-1020-0

Lee, J., Lee, S., & Young, J. P. W. (2008). Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology*, *65*(2), 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x

Lin, Y., Ye, Y., Hu, Y., & Shi, H. (2019). The variation in microbial community structure under different heavy metal contamination levels in paddy soils. *Ecotoxicology and Environmental Safety*, *180*, 557–564. https://doi.org/10.1016/j.ecoenv.2019.05.057

Liu, J., Sui, Y., Yu, Z., Shi, Y., Chu, H., Jin, J., Liu, X., & Wang, G. (2014). *High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China*. https://doi.org/10.1016/j.soilbio.2013.12.014

López-Bucio, J., Pelagio-Flores, R., & Herrera-Estrella, A. (2015). Trichoderma as biostimulant: Exploiting the multilevel properties of a plant beneficial fungus. *Scientia Horticulturae*, *196*(September), 109–123. https://doi.org/10.1016/j.scienta.2015.08.043

Lüneberg, K., Schneider, D., Siebe, C., & Daniel, R. (2018). Drylands soil bacterial community is affected by land use change and different irrigation practices in the Mezquital Valley, Mexico. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-018-19743-x

Magoč, T., Magoč, M., & Salzberg, S. L. (2011). *FLASH: fast length adjustment of short reads to improve genome assemblies*. *27*(21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Mahmoudi, N., Cruz, C., Mahdhi, M., Mars, M., & Caeiro, M. F. (2019). Arbuscular mycorrhizal fungi in soil, roots and rhizosphere of Medicago truncatula: Diversity and heterogeneity under semi-arid conditions. *PeerJ*, *2019*(3). https://doi.org/10.7717/peerj.6401

Majid, S. A., Graw, M. F., Chatziefthimiou, A. D., Nguyen, H., Richer, R., Louge, M., Sultan, A. A., Schloss, P., & Hay, A. G. (2016). Microbial characterization of

Qatari Barchans and Dunes. *PLoS ONE*, *11*(9), 1–22. https://doi.org/10.1371/journal.pone.0161836

Mandal, A., & Sathyaseelan, N. (2012). Impact of Climate Change on Soil Biodiversity - a Review. *Agricultural Reviews*, *33*(4), 283–292.

Maron, P.-A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., & Ranjard, L. (2018). High Microbial Diversity Promotes Soil Ecosystem Functioning. *Environmental Microbiology*, *84*(9), 1–13.

Masson, P., Dalix, T., & Bussière, S. (2010). Determination of major and trace elements in plant samples by inductively coupled plasma-mass spectrometry. *Communications in Soil Science and Plant Analysis*, *41*(3), 231–243. https://doi.org/10.1080/00103620903460757

McGeehan, S. L., & Naylor, D. V. (1988). Automated instrumental analysis of carbon and nitrogen in plant and soil samples. *Communications in Soil Science and Plant Analysis*, *19*(4), 493–505. https://doi.org/10.1080/00103628809367953

McHugh, T. A., Compson, Z., Gestel, N. van, Hayer, M., Ballard, L., Haverty, M., Hines, J., Irvine, N., Krassner, D., Lyons, T., Musta, E. J., Schiff, M., Zint, P., & Schwartz, E. (2017). Climate controls prokaryotic community composition in desert soils of the southwestern United States. *FEMS Microbiology Ecology*, *93*(10), 1–13. https://doi.org/10.1093/femsec/fix116

Mendoza, R., Cabello, M., Anchorena, J., García, I., & Marbán, L. (2011). Soil parameters and host plants associated with arbuscular mycorrhizae in the grazed Magellanic steppe of Tierra del Fuego. *Agriculture, Ecosystems and Environment*, *140*(3–4), 411–418. https://doi.org/10.1016/j.agee.2011.01.004

Miranda, J. C. C., & Harris, P. J. (1994). Effects of soil phosphorus on spore

germination and hyphal growth of arbuscular mycorrhizal fungi. *New Phytologist*, *128*(1), 103–108. https://doi.org/10.1111/j.1469-8137.1994.tb03992.x

Moons, A. (2003). Osgstu3 and osgtu4, encoding tau class glutathione S-transferases, are heavy metal- and hypoxic stress-induced and differentially salt stress-responsive in rice roots. *FEBS Letters*, *553*(3), 427–432. https://doi.org/10.1016/S0014-5793(03)01077-9

Morton, J. B. (1986). Three New Species of Acaulospora (Endogonaceae) From High Aluminum, Low Ph Soils in West Virginia . *Mycologia*, *78*(4), 641–648. https://doi.org/10.1080/00275514.1986.12025300

Neilson, J. W., Califf, K., Cardona, C., Copeland, A., van Treuren, W., Josephson, K. L., Knight, R., Gilbert, J. A., Quade, J., Caporaso, J. G., Maier, R. M., Califf, K., van Treuren, W., Gilbert, J. A., Quade, J., Neilson, J. W., Knight, R., Cardona, C., Caporaso, J. G., … Maier, R. M. (2017). Significant Impacts of Increasing Aridity on the Arid Soil Microbiome. *MSystems*, *2*(3), 1–15. https://doi.org/10.1128/msystems.00195-16

Nilsson, R. H., Ryberg, M., Abarenkov, K., Sjökvist, E., & Kristiansson, E. (2009). The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*, *296*(1), 97–101. https://doi.org/10.1111/j.1574-6968.2009.01618.x

Norton, J., Abdul Majid, S., Allan, D., Al Safran, M., Böer, B., & Richer, R. (Renee A. . (2009). *An illustrated checklist of the flora of Qatar*.

O'Donnell, K., Lutzoni, F. M., Ward, T. J., & Benny, G. L. (2001). Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. *Mycologia*, *93*(2), 286–296. https://doi.org/10.2307/3761650

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., & Sieverding, E. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry*, *42*(5), 724–738. https://doi.org/10.1016/j.soilbio.2010.01.006

Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., & Colpaert, J. V. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0097629

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., Reier, Ü., & Zobel, M. (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist*, *188*(1), 223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

Öpik, Maarja, & Davison, J. (2016). Uniting species- and community-oriented approaches to understand arbuscular mycorrhizal fungal diversity. *Fungal Ecology*, *24*, 106–113. https://doi.org/10.1016/j.funeco.2016.07.005

Oren, A. (2008). Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Systems*, *4*(1), 2. https://doi.org/10.1186/1746-1448-4-2

Osuji, L. C., & Onojake, C. M. (2004). Trace heavy metals associated with crude oil: A case study of ebocha-8 oil-spill-polluted site in niger delta, Nigeria. *Chemistry and Biodiversity*, *1*(11), 1708–1715. https://doi.org/10.1002/cbdv.200490129

Otsing, E., Barantal, S., Anslan, S., Koricheva, J., & Tedersoo, L. (2018). Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. *Soil Biology and Biochemistry*, *125*(July), 328–339. https://doi.org/10.1016/j.soilbio.2018.08.006

Oyediran, O. K., Kumar, A. G., & Neelam, J. (2018). Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Tomato Grown in Arid and Semi-arid Regions of Indian Desert. *Asian Journal of Agricultural Research*, *12*(1), 10–18. https://doi.org/10.3923/ajar.2018.10.18

Pan, X., Zhang, S., Zhong, Q., Gong, G., Wang, G., Guo, X., & Xu, X. (2020). Effects of soil chemical properties and fractions of Pb, Cd, and Zn on bacterial and fungal communities. *Science of the Total Environment*, *715*, 136904. https://doi.org/10.1016/j.scitotenv.2020.136904

Patriquin, D. G., Blaikie, H., Patriquin, M. J., & Yang, C. (1993). On-farm measurements of pH, electrical conductivity and nitrate in soil extracts for monitoring coupling and decoupling of nutrient cycles. *Biological Agriculture and Horticulture*, *9*(3), 231–272. https://doi.org/10.1080/01448765.1993.9754638

Pinto, U., Maheshwari, B. L., & Grewal, H. S. (2010). Effects of greywater irrigation on plant growth, water use and soil properties. *Resources, Conservation and Recycling*, *54*(7), 429–435. https://doi.org/10.1016/j.resconrec.2009.09.007

Pozo, M. J., & Azcón-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. In *Current Opinion in Plant Biology* (Vol. 10, Issue 4, pp. 393–398). https://doi.org/10.1016/j.pbi.2007.05.004

Querejeta, J. I., Allen, M. F., Caravaca, F., & Roldán, A. (2006). Differential modulation of host plant δ13C and δ18O by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. *New Phytologist*, *169*(2), 379–387. https://doi.org/10.1111/j.1469-8137.2005.01599.x

Rengasamy, P. (2009). *Chemistry Soil pH - South Australia*.

Reynolds, J. F., Stafford Smith, D. M., Lambin, E. F., Turner, B. L., Mortimore, M., Batterbury, S. P. J., Downing, T. E., Dowlatabadi, H., Fernández, R. J., Herrick,

J. E., Huber-Sannwald, E., Jiang, H., Leemans, R., Lynam, T., Maestre, F. T., Ayarza, M., & Walker, B. (2007). Ecology: Global desertification: Building a science for dryland development. *Science*, *316*(5826), 847–851. https://doi.org/10.1126/science.1131634

Rhoads, A., & Au, K. F. (2015). PacBio Sequencing and Its Applications. *Genomics, Proteomics and Bioinformatics*, *13*(5), 278–289. https://doi.org/10.1016/j.gpb.2015.08.002

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*(1), 41–53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *2016*(10), 1–22. https://doi.org/10.7717/peerj.2584

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., Pascale, S. De, Bonini, P., & Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae*, *196*, 91–108. https://doi.org/10.1016/j.scienta.2015.09.002

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME Journal*, *4*(10), 1340–1351. https://doi.org/10.1038/ismej.2010.58

Schlaeppi, K., Bender, S. F., Mascher, F., Russo, G., Patrignani, A., Camenzind, T., Hempel, S., Rillig, M. C., & van der Heijden, M. G. A. (2016). High-resolution community profiling of arbuscular mycorrhizal fungi. *New Phytologist*, *212*(3), 780–791. https://doi.org/10.1111/nph.14070

Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S., & Schloter, M. (2017). Analysis

of soil microbial communities based on amplicon sequencing of marker genes. *Biology and Fertility of Soils*, *53*(5), 485–489. https://doi.org/10.1007/s00374-017-1205-1

Schüβler, A., Schwarzott, D., & Walker, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research*, *105*(12), 1413–1421. http://www.sciencedirect.com/science/article/pii/S0953756208620262

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, *21*(8), 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Siles, J. A., Cajthaml, T., Hernández, P., Pérez-Mendoza, D., García-Romera, I., & Sampedro, I. (2015). Shifts in Soil Chemical Properties and Bacterial Communities Responding to Biotransformed Dry Olive Residue Used as Organic Amendment. *Microbial Ecology*, *70*(1), 231–243. https://doi.org/10.1007/s00248-014-0552-9

Smith, A. P., DeRidder, B. P., Guo, W. J., Seeley, E. H., Regnier, F. E., & Goldsbrough, P. B. (2004). Proteomic analysis of Arabidopsis glutathione S-transferases from benoxacor- and copper-treated seedlings. *Journal of Biological Chemistry*, *279*(25), 26098–26104. https://doi.org/10.1074/jbc.M402807200

Smith, C. (1997). A revised six-kingdom system of life. *Biological Reviews*, *73*(3), 203–266. https://doi.org/10.1111/j.1469-185X.1998.tb00030.x

Song, J., Shen, Q., Wang, L., Qiu, G., Shi, J., Xu, J., Brookes, P. C., & Liu, X. (2018). Effects of Cd, Cu, Zn and their combined action on microbial biomass and bacterial community structure. *Environmental Pollution*, *243*(Pt A), 510–518. https://doi.org/10.1016/j.envpol.2018.09.011

Soudzilovskaia, N. A., Douma, J. C., Akhmetzhanova, A. A., van Bodegom, P. M., Cornwell, W. K., Moens, E. J., Treseder, K. K., Tibbett, M., Wang, Y.-P., & Cornelissen, J. H. C. (2015). Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. *Global Ecology and Biogeography*, *24*(3), 371–382. https://doi.org/10.1111/geb.12272

Tandon. (2005). *Methods of analysis of soils, plants, waters, fertilisers & organic manures*. Fertiliser Development and Consultation Organisation.

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, R. H., Hildebrand, F., Bork, P., & Abarenkov, K. (2015). Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys*, *10*, 1–43. https://doi.org/10.3897/mycokeys.10.4852

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yurou, N., Wijesundera, R., Ruiz, L., Vasco-Palacios, A., & Thu, P. Q. (2014). Global diversity and geography of soil fungi. *Science (New York, N.Y.)*, *346*(6213), 1052–1053. https://doi.org/10.1126/science.aaa1185

Tedersoo, L., & Lindahl, B. (2016). Fungal identification biases in microbiome projects. *Environmental Microbiology Reports*, *8*(5), 774–779. https://doi.org/10.1111/1758-2229.12438

Tedersoo, L., Tooming-Klunderud, A., & Anslan, S. (2018). PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. *New Phytologist*, *217*(3), 1370–1385. https://doi.org/10.1111/nph.14776

Tian, Q., Taniguchi, T., Shi, W. Y., Li, G., Yamanaka, N., & Du, S. (2017). Land-use types and soil chemical properties influence soil microbial communities in the semiarid Loess Plateau region in China. *Scientific Reports*, *7*(October 2016), 1–9.

https://doi.org/10.1038/srep45289

Tighe, M., Lockwood, P., Wilson, S., & Lisle, L. (2004). Comparison of digestion methods for ICP-OES analysis of a wide range of analytes in heavy metal contaminated soil samples with specific reference to arsenic and antimony. *Communications in Soil Science and Plant Analysis*, *35*(9–10), 1369–1385. https://doi.org/10.1081/CSS-120037552

Timmer, L. W., & Leyden, R. F. (1980). The Relationship of Mycorrhizal Infection to Phosphorous-induced Copper Deficiency in Sour Orange Seedlings. *New Phytologist*, *85*(1), 15–23. https://doi.org/10.1111/j.1469-8137.1980.tb04443.x

Toljander, J. F., Santos-González, J. C., Tehler, A., & Finlay, R. D. (2008). Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. *FEMS Microbiology Ecology*, *65*(2), 323–338. https://doi.org/10.1111/j.1574-6941.2008.00512.x

Torrecillas, E., Alguacil, M. del M., Roldán, A., Díaz, G., Montesinos-Navarro, A., & Torres, M. P. (2014). Modularity reveals the tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gypsum soils. *Applied and Environmental Microbiology*, *80*(17), 5457–5466. https://doi.org/10.1128/AEM.01358-14

Treseder, K. K., & Lennon, J. T. (2015). Fungal Traits That Drive Ecosystem Dynamics on Land. *Microbiology and Molecular Biology Reviews*, *79*(2), 243–262. https://doi.org/10.1128/mmbr.00001-15

Tresner, H. D., & Hayes, J. A. (1971). Sodium chloride tolerance of terrestrial fungi. *Applied Microbiology*, *22*(2), 210–213. https://doi.org/10.1128/AEM.22.2.210-213.1971

Tripathy, S., Bhattacharyya, P., Mohapatra, R., Som, A., & Chowdhury, D. (2014).

Influence of different fractions of heavy metals on microbial ecophysiological indicators and enzyme activities in century old municipal solid waste amended soil. *Ecological Engineering*, *70*, 25–34. https://doi.org/10.1016/j.ecoleng.2014.04.013

Trivedi, P., Delgado-Baquerizo, M., Anderson, I. C., & Singh, B. K. (2016). Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators. *Frontiers in Plant Science*, *7*(July), 1–13. https://doi.org/10.3389/fpls.2016.00990

United Nations. (2011). *Global Drylands*.

Van Der Heijden, M. G. A., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(3), 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

Van Elsas, J., Garbeva, P., & Salles, J. (2002). Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. In *Biodegradation* (Vol. 13).

Vasar, M., Andreson, R., Davison, J., Jairus, T., Moora, M., Remm, M., Young, J. P. W., Zobel, M., & Öpik, M. (2017). Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, *27*(8), 761–773. https://doi.org/10.1007/s00572-017-0791-y

Villalobos, F. J., Mateos, L., Quemada, M., Delgado, A., & Fereres, E. (2016). Control of Salinity. In F. J. Villalobos & E. Fereres (Eds.), *Principles of Agronomy for*

Sustainable Agriculture (pp. 295–320). Springer International Publishing. https://doi.org/10.1007/978-3-319-46116-8_22

Vogel-Mikuš, K., Pongrac, P., Kump, P., Nečemer, M., & Regvar, M. (2006). Colonisation of a Zn, Cd and Pb hyperaccumulator Thlaspi praecox Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environmental Pollution*, *139*(2), 362–371. https://doi.org/10.1016/j.envpol.2005.05.005

Vyas, D., & Kumar Gupta, R. (2014). *Effect of edaphic factors on the diversity of VAM fungi*. www.tropicalplantresearch.com

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Walker, C., & Vestberg, M. (1998). Synonymy Amongst the Arbuscular Mycorrhizal Fungi : Glomus claroideum, G. maculosum, G. multisubstenum and G. fistulosum. In *Annals of Botany* (Vol. 82).

Wang, J., Zhang, T., Li, L., Li, J., Feng, Y., & Lu, Q. (2017). The patterns and drivers of bacterial and fungal β-diversity in a typical dryland ecosystem of northwest China. *Frontiers in Microbiology*, *8*(NOV), 1–11. https://doi.org/10.3389/fmicb.2017.02126

Wang, R., Zhang, H., Sun, L., Qi, G., Chen, S., & Zhao, X. (2017). Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-00472-6

Wang, Y., Zhang, L., & Haimiti, Y. (2015). Study on Spatial Variability of Soil

Nutrients in Ebinur Lake Wetlands in China. *Journal of Coastal Research*, 59–63. https://doi.org/10.2112/SI73-011.1

Wang, Z., Liu, L., Chen, Q., Wen, X., & Liao, Y. (2016). Conservation tillage increases soil bacterial diversity in the dryland of northern China. *Agronomy for Sustainable Development*, *36*(2), 1–9. https://doi.org/10.1007/s13593-016-0366-x

Weissenhorn, I., Leyval, C., & Berthelin, J. (1993). Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. In *Plant and Soil* (Vol. 157).

Wood, E. D., Armstrong, F. A. J., & Richards, F. A. (1967). Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom*, *47*(1), 23–31. https://doi.org/10.1017/S002531540003352X

Wu, B., Hogetsu, T., Isobe, K., & Ishii, R. (2007). Community structure of arbuscular mycorrhizal fungi in a primary successional volcanic desert on the southeast slope of Mount Fuji. *Mycorrhiza*, *17*(6), 495–506. https://doi.org/10.1007/s00572-007-0114-9

Wu, Z., Liu, Q., Li, Z., Cheng, W., Sun, J., Guo, Z., Li, Y., Zhou, J., Meng, D., Li, H., Lei, P., & Yin, H. (2018). Environmental factors shaping the diversity of bacterial communities that promote rice production. *BMC Microbiology*, *18*(1), 1–11. https://doi.org/10.1186/s12866-018-1174-z

Xue, P. P., Carrillo, Y., Pino, V., Minasny, B., & McBratney, A. B. (2018). Soil Properties Drive Microbial Community Structure in a Large Scale Transect in South Eastern Australia. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-30005-8

Yan, N., Marschner, P., Cao, W., Zuo, C., & Qin, W. (2015). Influence of salinity and water content on soil microorganisms. *International Soil and Water Conservation*

Research, *3*(4), 316–323. https://doi.org/10.1016/j.iswcr.2015.11.003

Yan, X., Liu, M., & Zhong, J. (2018). *How Human Activities Affect Heavy Metal Contamination of Soil and Sediment in a Long-Term Reclaimed Area of the Liaohe River Delta*. 1–19. https://doi.org/10.3390/su10020338

Zarei, M., Hempel, S., Wubet, T., Schäfer, T., Savaghebi, G., Jouzani, G. S., Nekouei, M. K., & Buscot, F. (2010). Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. *Environmental Pollution*, *158*(8), 2757–2765. https://doi.org/10.1016/j.envpol.2010.04.017

Zhang, C., Liu, G., Song, Z., Qu, D., Fang, L., & Deng, L. (2017). *Natural succession on abandoned cropland effectively decreases the soil erodibility and improves the fungal diversity*. *27*(7), 2142–2154.

Zhang, H., Wei, S., Hu, W., Xiao, L., & Tang, M. (2017). Arbuscular mycorrhizal fungus rhizophagus irregularis increased potassium content and expression of genes encoding potassium channels in lycium barbarum. *Frontiers in Plant Science*, *8*, 440–440. https://doi.org/10.3389/fpls.2017.00440

Zhao, H., Li, X., Zhang, Z., Zhao, Y., Yang, J., & Zhu, Y. (2017). Species diversity and drivers of arbuscular mycorrhizal fungal communities in a semi-arid mountain in China. *PeerJ*, *2017*(12). https://doi.org/10.7717/peerj.4155

APPENDIX A: SUMMARY DATA OF SOIL %TC ANALYSIS

Table 25. Areas of each composite soil sample and calculated %TC according to extrapolated data from Glycine standard calibration

Composite sample	Wt. sample, mg	Area	Wt. C, mg	%TC (as measured)
1	263.6	2480383.65	13.7720	5.22
2	340.2	6612453.49	36.9338	10.86
3	261.2	4330044.91	24.1401	9.24
4	259.8	2940875.69	16.3532	6.29
5	268.1	2850564.92	15.8470	5.91
6	265.8	2171258.60	12.0392	4.53
7	272.5	2096626.07	11.6209	4.26
8	289.0	1668118.61	9.2189	3.19
9	275.9	1496329.74	8.2560	2.99
10	258.9	3211351.80	17.8694	6.90
11	259.4	2484420.26	13.7946	5.32
12	268.3	5306549.92	29.6137	11.04
13	309.3	5069522.27	28.2851	9.14
14	269.4	3037455.83	16.8946	6.27
15	272.5	2009122.29	11.1304	4.08
16	315.0	1391482.41	7.6683	2.43
17	261.2	1718676.73	9.5023	3.64
18	251.5	1772457.75	9.8038	3.90
19	255.3	1662337.71	9.1865	3.60

APPENDIX B: SUMMARY DATA OF SOIL %TN ANALYSIS

Table 26. Areas of each composite soil sample and calculated %TN according to extrapolated data from Glycine standard calibration

Composite sample	Wt. sample, mg	Area	Wt. N, mg	%TN (as measured)
1	263.6	14918.59	-0.1481	NEG
2	340.2	18010.88	0.0475	0.014
3	261.2	13726.68	-0.2235	NEG
4	259.8	14522.83	-0.1732	NEG
5	268.1	14352.94	-0.1839	NEG
6	265.8	15815.14	-0.0914	NEG
7	272.5	14951.41	-0.1460	NEG
8	289.0	12614.98	-0.2938	NEG
9	275.9	13134.65	-0.2610	NEG
10	258.9	16703.12	-0.0352	NEG
11	259.4	13886.29	-0.2134	NEG
12	268.3	16472.24	-0.0498	NEG
13	309.3	12744.38	-0.2857	NEG
14	269.4	12976.21	-0.2710	NEG
15	272.5	13272.63	-0.2522	NEG
16	315.0	12629.87	-0.2929	NEG
17	261.2	13170.26	-0.2587	NEG
18	251.5	12773.36	-0.2838	NEG
19	255.3	13163.68	-0.2591	NEG

APPENDIX C: SUMMARY DATA OF SOIL NITRITE

Table 27. Absorbance values of each composite soil sample and calculated soil nitrite concentration according to extrapolated data of nitrite standard calibration

Sample	Wt. (g)	Abs.	Conc. µg NO_2-N/l	Amount of N, µg	Conc. µg NO_2-N/kg soil	Conc. mg NO_2^-/kg soil
1	2.01	1.63	480.68	24.03	11.94	39.20
2	2.03	1.56	461.26	23.06	11.36	37.32
3	2.09	1.60	470.97	23.55	11.29	37.08
4	2.00	1.54	455.09	22.75	11.36	37.33
5	2.04	1.50	443.62	22.18	10.88	35.75
6	2.01	1.55	455.97	22.80	11.33	37.21
7	2.00	1.59	469.21	23.46	11.71	38.45
8	2.27	1.55	458.62	22.93	10.10	33.18
9	2.03	1.52	449.79	22.49	11.06	36.33
10	2.07	1.48	437.44	21.87	10.57	34.71
11	2.08	1.59	469.21	23.46	11.30	37.10
12	2.13	1.57	463.03	23.15	10.86	35.66
13	2.11	1.57	463.91	23.20	10.99	36.11
14	2.11	1.56	459.50	22.98	10.88	35.75
15	2.06	1.59	468.32	23.42	11.39	37.41
16	2.08	1.49	438.32	21.92	10.54	34.62
17	2.18	1.59	469.21	23.46	10.77	35.36
18	2.02	1.63	480.68	24.03	11.91	39.12
19	2.03	1.58	466.56	23.33	11.47	37.67

APPENDIX D: SUMMARY DATA OF SOIL NITRATE

Table 28. Absorbance values of each composite soil sample and calculated soil nitrate concentration according to extrapolated data of nitrate standard calibration

Sample	Wt. (g)	Abs.	Conc. µg NO_3-N/l	Amount of N, µg	Conc. µg(NO_3 + NO_2)-N/kg soil	Conc. µgNO_3-N/kg soil	Conc. mg NO_3^-/kg soil
1	2.01	1.78	1039.41	51.97	25.81	13.87	61.44
2	2.03	1.98	1157.06	57.85	28.50	17.14	75.92
3	2.09	2.95	1730.59	86.53	41.49	30.20	133.74
4	2.00	2.50	1465.88	73.29	36.60	25.24	111.79
5	2.04	2.15	1260.00	63.00	30.91	20.03	88.71
6	2.01	1.90	1112.94	55.65	27.65	16.32	72.29
7	2.00	2.25	1318.82	65.94	32.90	21.20	93.88
8	2.27	2.38	1392.35	69.62	30.67	20.57	91.10
9	2.03	2.08	1215.88	60.79	29.90	18.84	83.43
10	2.07	10.40	6127.65	306.38	148.05	137.48	608.88
11	2.08	2.00	1171.76	58.59	28.21	16.91	74.91
12	2.13	1.58	921.76	46.09	21.61	10.76	47.63
13	2.11	1.83	1068.82	53.44	25.33	14.34	63.49
14	2.11	2.55	1495.29	74.76	35.42	24.53	108.66
15	2.06	3.00	1760.00	88.00	42.81	31.42	139.14
16	2.08	1.98	1157.06	57.85	27.82	17.28	76.54
17	2.18	3.60	2112.94	105.65	48.48	37.72	167.05
18	2.02	2.60	1524.71	76.24	37.78	25.87	114.57
19	2.03	1.07	621.76	31.09	15.28	3.82	16.90

APPENDIX E: TEST OF NORMALITY

Table 29. Shapiro-Wilk normality test was used to evaluate the distribution of each variable at 95% confidence interval. Bold values indicate that the sig. values < 0.05 and thus, showed that the data was not normally distributed. These datasets were normalized by logarithmic transformation

Locations	df	pH		EC		Salinity		TDS		NO_2^-		NO_3^-		TC	
		Statistic	Sig.	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.
AlSakhama	3	0.842	0.220	1.000	1.000	0.750	**0.000**	0.942	0.537	0.871	0.298	0.980	0.731	0.815	0.150
AlKhor	3	0.999	0.952	0.999	0.942	0.750	**0.000**	0.871	0.298	0.750	**0.000**	0.952	0.579	0.837	0.206
AlThakira	3	0.923	0.463	0.964	0.637	0.750	**0.000**	1.000	0.967	0.991	0.817	0.969	0.664	0.893	0.363
Lusail	3	0.987	0.780	0.914	0.433			0.923	0.463	0.846	0.230	1.000	0.987	0.774	0.054
QU1	3	0.774	0.054	0.992	0.831	0.750	**0.000**	0.923	0.463	0.987	0.780	0.988	0.788	0.848	0.234
QU2	3	0.923	0.463	0.987	0.780	0.750	**0.000**	0.925	0.470	0.832	0.195	0.942	0.535	0.994	0.856
AlGhuwayriyah	3	0.923	0.463	0.999	0.952	0.750	**0.000**	1.000	1.000	0.985	0.762	1.000	0.960	0.818	0.157
AlNu'man	3	0.949	0.567	0.996	0.878			0.750	**0.000**	0.927	0.478	0.999	0.934	0.805	0.127
AlJumayliyah	3	1.000	1.000	0.987	0.780	0.750	**0.000**	0.855	0.253	0.990	0.808	0.999	0.948	0.923	0.463
Khawzan	3	0.964	0.637	0.980	0.730	0.750	**0.000**	0.881	0.328	0.996	0.881	0.890	0.354	0.834	0.198
AlKharrara1	3	0.818	0.157	0.789	0.089			0.936	0.510	0.993	0.843	0.981	0.739	0.889	0.352
AlKharrara2	3	0.842	0.220	0.964	0.637	1.000	1.000	0.996	0.878	0.995	0.866	0.969	0.661	0.957	0.600
AlRayyan	3	0.824	0.174	0.949	0.567	0.964	0.637	0.842	0.220	0.947	0.555	0.851	0.244	0.964	0.637
AlKharsaah1	3	1.000	1.000	0.838	0.209	0.750	**0.000**	0.893	0.363	0.877	0.316	0.945	0.548	0.910	0.417
AlKharsaah2	3	0.750	**0.000**	0.923	0.463	0.750	**0.000**	0.871	0.298	0.891	0.357	0.809	0.137	0.881	0.328
Dukhan	3	0.824	0.174	0.883	0.334			0.871	0.298	0.999	0.949	0.996	0.882	0.987	0.780
North Qatar1	3	0.987	0.780	0.987	0.780	0.750	**0.000**	0.923	0.463	0.944	0.544	0.992	0.833	0.947	0.554
North Qatar2	3	1.000	1.000	0.990	0.812	0.750	**0.000**	0.855	0.253	0.828	0.182	1.000	0.976	0.928	0.480
AlKiranah	3	0.832	0.194	0.929	0.484			0.871	0.298	0.831	0.192	0.784	0.078	0.977	0.708

APPENDIX F: TAGGED FORWARD AND REVERSE AMF-SPECIFIC PRIMER SEQUENCES

Table 30. Library codes (QU01AMF and QU02AMF) of each DNA sample with its corresponding tagged forward and reverse AMF-specific primer sequences

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
11(1)/13(20)	Wanda_201	ACAACACTCCGACAGCCGCGGTA ATTCCAGCT	AML2_201	ACAACACTCCGAGAACCCAAACAC TTTGGTTTCC
11(2)/14(1)	Wanda_202	ACAAGTGCTGCTCAGCCGCGGTA ATTCCAGCT	AML2_202	ACAAGTGCTGCTGAACCCAAACAC TTTGGTTTCC
11(3)/14(2)	Wanda_203	ACACAGTCCTGACAGCCGCGGTA ATTCCAGCT	AML2_203	ACACAGTCCTGAGAACCCAAACAC TTTGGTTTCC
11(4)/14(3)	Wanda_204	ACACCAACACCACAGCCGCGGTA ATTCCAGCT	AML2_204	ACACCAACACCAGAACCCAAACAC TTTGGTTTCC
11(5)/14(4)	Wanda_205	ACACCGCACAATCAGCCGCGGTA ATTCCAGCT	AML2_205	ACACCGCACAATGAACCCAAACAC TTTGGTTTCC
11(6)/14(5)	Wanda_206	ACACTTCGGCAACAGCCGCGGTA ATTCCAGCT	AML2_206	ACACTTCGGCAAGAACCCAAACAC TTTGGTTTCC
11(7)/14(6)	Wanda_208	ACAGTGCGTCCTCAGCCGCGGTA ATTCCAGCT	AML2_208	ACAGTGCGTCCTGAACCCAAACAC TTTGGTTTCC
11(8)/14(7)	Wanda_209	ACATACTGAGCACAGCCGCGGTA ATTCCAGCT	AML2_209	ACATACTGAGCAGAACCCAAACAC TTTGGTTTCC

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
11(9)/14(8)	Wanda_210	ACATCTAGCAGACAGCCGCGGTAATTCCAGCT	AML2_210	ACATCTAGCAGAGAACCCAAACACTTTGGTTTCC
11(10)/14(9)	Wanda_211	ACATTGAAGCGTCAGCCGCGGTAATTCCAGCT	AML2_211	ACATTGAAGCGTGAACCCAAACACTTTGGTTTCC
11(11)/14(10)	Wanda_212	ACCACACGTAGTCAGCCGCGGTAATTCCAGCT	AML2_212	ACCACACGTAGTGAACCCAAACACTTTGGTTTCC
11(12)/14(11)	Wanda_213	ACCACGATGCTACAGCCGCGGTAATTCCAGCT	AML2_213	ACCACGATGCTAGAACCCAAACACTTTGGTTTCC
11(13)/14(12)	Wanda_214	ACCAGCTCAGATCAGCCGCGGTAATTCCAGCT	AML2_214	ACCAGCTCAGATGAACCCAAACACTTTGGTTTCC
11(14)/14(13)	Wanda_215	ACCAGTGACTCACAGCCGCGGTAATTCCAGCT	AML2_215	ACCAGTGACTCAGAACCCAAACACTTTGGTTTCC
11(15)/14(14)	Wanda_216	ACCATCCAACGACAGCCGCGGTAATTCCAGCT	AML2_216	ACCATCCAACGAGAACCCAAACACTTTGGTTTCC
11(16)/14(15)	Wanda_217	ACCGACGCTTGTCAGCCGCGGTAATTCCAGCT	AML2_217	ACCGACGCTTGTGAACCCAAACACTTTGGTTTCC
11(17)/14(16)	Wanda_218	ACCGATTAGGTACAGCCGCGGTAATTCCAGCT	AML2_218	ACCGATTAGGTAGAACCCAAACACTTTGGTTTCC
11(18)/14(17)	Wanda_219	ACCGGAGTAGGACAGCCGCGGTAATTCCAGCT	AML2_219	ACCGGAGTAGGAGAACCCAAACACTTTGGTTTCC
11(19)/14(18)	Wanda_220	ACCGTAAGACATCAGCCGCGGTAATTCCAGCT	AML2_220	ACCGTAAGACATGAACCCAAACACTTTGGTTTCC
11(20)/14(19)	Wanda_221	ACCGTGCTCACACAGCCGCGGTAATTCCAGCT	AML2_221	ACCGTGCTCACAGAACCCAAACACTTTGGTTTCC

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
12(1)/14(20)	Wanda_222	ACCTACTTGTCTCAGCCGCGGTAATTCCAGCT	AML2_222	ACCTACTTGTCTGAACCCAAACACTTTGGTTTCC
12(2)/15(1)	Wanda_223	ACCTATGGTGAACAGCCGCGGTAATTCCAGCT	AML2_223	ACCTATGGTGAAGAACCCAAACACTTTGGTTTCC
12(3)/15(2)	Wanda_224	ACCTCTATTCGTCAGCCGCGGTAATTCCAGCT	AML2_224	ACCTCTATTCGTGAACCCAAACACTTTGGTTTCC
12(4)/15(3)	Wanda_225	ACCTGATCCGCACAGCCGCGGTAATTCCAGCT	AML2_225	ACCTGATCCGCAGAACCCAAACACTTTGGTTTCC
12(5)/15(4)	Wanda_226	ACCTTACACCTTCAGCCGCGGTAATTCCAGCT	AML2_226	ACCTTACACCTTGAACCCAAACACTTTGGTTTCC
12(6)/15(5)	Wanda_227	ACCTTGACAAGACAGCCGCGGTAATTCCAGCT	AML2_227	ACCTTGACAAGAGAACCCAAACACTTTGGTTTCC
12(7)/15(6)	Wanda_228	ACGACCTACGCTCAGCCGCGGTAATTCCAGCT	AML2_228	ACGACCTACGCTGAACCCAAACACTTTGGTTTCC
12(8)/15(7)	Wanda_229	ACGACTGCATAACAGCCGCGGTAATTCCAGCT	AML2_229	ACGACTGCATAAGAACCCAAACACTTTGGTTTCC
12(9)/15(8)	Wanda_230	ACGAGACTGATTCAGCCGCGGTAATTCCAGCT	AML2_230	ACGAGACTGATTGAACCCAAACACTTTGGTTTCC
12(10)/15(9)	Wanda_231	ACGAGGAGTCGACAGCCGCGGTAATTCCAGCT	AML2_231	ACGAGGAGTCGAGAACCCAAACACTTTGGTTTCC
12(11)/15(10)	Wanda_232	ACGATATGGTCACAGCCGCGGTAATTCCAGCT	AML2_232	ACGATATGGTCAGAACCCAAACACTTTGGTTTCC
12(12)/15(11)	Wanda_233	ACGATGGTTGATCAGCCGCGGTAATTCCAGCT	AML2_233	ACGATGGTTGATGAACCCAAACACTTTGGTTTCC

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
12(13)/15(12)	Wanda_234	ACGCACATACAACAGCCGCGGTAATTCCAGCT	AML2_234	ACGCACATACAAGAACCCAAACACTTTGGTTTCC
12(14)/15(13)	Wanda_235	ACGCATCGCACTCAGCCGCGGTAATTCCAGCT	AML2_235	ACGCATCGCACTGAACCCAAACACTTTGGTTTCC
12(15)/15(14)	Wanda_236	ACGCGAACTAATCAGCCGCGGTAATTCCAGCT	AML2_236	ACGCGAACTAATGAACCCAAACACTTTGGTTTCC
12(16)/15(15)	Wanda_237	ACGCTAGATTGACAGCCGCGGTAATTCCAGCT	AML2_237	ACGCTAGATTGAGAACCCAAACACTTTGGTTTCC
12(17)/15(16)	Wanda_238	ACGCTGTCGGTTCAGCCGCGGTAATTCCAGCT	AML2_238	ACGCTGTCGGTTGAACCCAAACACTTTGGTTTCC
12(18)/15(17)	Wanda_239	ACGGCGTTATGTCAGCCGCGGTAATTCCAGCT	AML2_239	ACGGCGTTATGTGAACCCAAACACTTTGGTTTCC
12(19)/15(18)	Wanda_240	ACGTAACCACGTCAGCCGCGGTAATTCCAGCT	AML2_240	ACGTAACCACGTGAACCCAAACACTTTGGTTTCC
12(20)/15(19)	Wanda_241	ACGTATTCGAAGCAGCCGCGGTAATTCCAGCT	AML2_241	ACGTATTCGAAGGAACCCAAACACTTTGGTTTCC
13(1)/15(20)	Wanda_242	ACGTGCCTTAGACAGCCGCGGTAATTCCAGCT	AML2_242	ACGTGCCTTAGAGAACCCAAACACTTTGGTTTCC
13(2)/COM1	Wanda_243	ACGTGTAGGCTTCAGCCGCGGTAATTCCAGCT	AML2_243	ACGTGTAGGCTTGAACCCAAACACTTTGGTTTCC
13(3)/COM2	Wanda_244	ACTAATACGCGACAGCCGCGGTAATTCCAGCT	AML2_244	ACTAATACGCGAGAACCCAAACACTTTGGTTTCC
13(4)/COM3	Wanda_245	ACTACCTCTTCACAGCCGCGGTAATTCCAGCT	AML2_245	ACTACCTCTTCAGAACCCAAACACTTTGGTTTCC

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
13(5)/COM4	Wanda_246	ACTACTGAGGATCAGCCGCGGTA ATTCCAGCT	AML2_246	ACTACTGAGGATGAACCCAAACAC TTTGGTTTCC
13(6)/COM5	Wanda_247	ACTAGACGACTACAGCCGCGGTA ATTCCAGCT	AML2_247	ACTAGACGACTAGAACCCAAACAC TTTGGTTTCC
13(7)/COM6	Wanda_248	ACTAGGATCAGTCAGCCGCGGTA ATTCCAGCT	AML2_248	ACTAGGATCAGTGAACCCAAACAC TTTGGTTTCC
13(8)/COM7	Wanda_249	ACTCACAGGAATCAGCCGCGGTA ATTCCAGCT	AML2_249	ACTCACAGGAATGAACCCAAACAC TTTGGTTTCC
13(9)/COM8	Wanda_250	ACTCATCTTCCACAGCCGCGGTA ATTCCAGCT	AML2_250	ACTCATCTTCCAGAACCCAAACACT TTGGTTTCC
13(10)/COM9	Wanda_251	ACTCCTTGTGTTCAGCCGCGGTAA TTCCAGCT	AML2_251	ACTCCTTGTGTTGAACCCAAACACT TTGGTTTCC
13(11)/COM10	Wanda_252	ACTCGGCCAACTCAGCCGCGGTA ATTCCAGCT	AML2_252	ACTCGGCCAACTGAACCCAAACAC TTTGGTTTCC
13(12)/COM11	Wanda_253	ACTCTAGCCGGTCAGCCGCGGTA ATTCCAGCT	AML2_253	ACTCTAGCCGGTGAACCCAAACAC TTTGGTTTCC
13(13)/COM12	Wanda_254	ACTGAGCTGCATCAGCCGCGGTA ATTCCAGCT	AML2_254	ACTGAGCTGCATGAACCCAAACAC TTTGGTTTCC
13(14)/COM13	Wanda_255	ACTGTACATGAGCAGCCGCGGTA ATTCCAGCT	AML2_255	ACTGTACATGAGGAACCCAAACAC TTTGGTTTCC
13(15)/COM14	Wanda_256	ACTGTCGCAGTACAGCCGCGGTA ATTCCAGCT	AML2_256	ACTGTCGCAGTAGAACCCAAACAC TTTGGTTTCC
13(16)/COM15	Wanda_257	ACTTCGGATGCACAGCCGCGGTA ATTCCAGCT	AML2_257	ACTTCGGATGCAGAACCCAAACAC TTTGGTTTCC

Sample	Tagged forward primer	Forward Primer Sequence	Tagged reverse primer	Reverse Primer Sequence
13(17)/COM16	Wanda_258	AGAACCGTCATACAGCCGCGGTAATTCCAGCT	AML2_258	AGAACCGTCATAGAACCCAAACACTTTGGTTTCC
13(18)/COM17	Wanda_259	AGAACTTGACGTCAGCCGCGGTAATTCCAGCT	AML2_259	AGAACTTGACGTGAACCCAAACACTTTGGTTTCC
13(19)/COM18	Wanda_260	AGAAGGCCTTATCAGCCGCGGTAATTCCAGCT	AML2_260	AGAAGGCCTTATGAACCCAAACACTTTGGTTTCC
COM19	Wanda_261	AGAATAGCGCTTCAGCCGCGGTAATTCCAGCT	AML2_261	AGAATAGCGCTTGAACCCAAACACTTTGGTTTCC
Positive	Wanda_207	ACAGACGACGGACAGCCGCGGTAATTCCAGCT	AML2_207	ACAGACGACGGAGAACCCAAACACTTTGGTTTCC
Negative	Wanda_262	AGATAGCTCGCTCAGCCGCGGTAATTCCAGCT	AML2_262	AGATAGCTCGCTGAACCCAAACACTTTGGTTTCC

APPENDIX G: INDICATOR INDEX ANALYSIS OF IDENTIFIED AMF FAMILIES

Table 31. Results of the indicator analysis (indicator value > 0.25) based on the abundance of AMF VTs for each identified family

Locations	AMF Family							
	Acaulospor-aceae	*Ambispor-aceae*	*Archaeospor-aceae*	*Claroideoglomer-aceae*	*Diversispora-ceae*	*Gigaspora-ceae*	*Glomerac-eae*	*Paraglomera-ceae*
AlSakhama	0	0	0	5	10	0	**84**	0
AlKhor	0	0	0	0	0	0	**59**	41
AlThakira	0	0	0	0	0	0	0	**100**
Lusail	0	0	0	32	11	0	**52**	5
QU field 1	0	0	0	6	8	0	**84**	2
AlGhuwayriyah	0	0	0	9	11	0	**80**	0
AlNu'man	0	0	0	5	5	0	**88**	2
AlJumayliyah	0	0	0	**48**	3	0	19	30
Khawzan	0	0	0	0	0	0	0	**100**
QU field 2	3	0	1	0	0	0	**85**	11
AlKharrara 1	0	0	0	14	22	0	**64**	0
AlKharrara 2	0	0	0	1	0	0	**99**	0
AlRayyan	0	0	0	11	4	0	**85**	0
AlKharsaah 1	0	0	0	18	4	0	**77**	1
AlKharsaah 2	0	0	0	7	1	0	**91**	0
Dukhan beach	0	0	0	0	0	0	**99**	1
South of Qatar 1	0	0	0	0	1	0	**97**	1
South of Qatar 2	0	0	0	0	0	0	**87**	13
AlKiranah	0	0	0	**100**	0	0	0	0
Indicator value	**0.0114**	**0.0004**	**0.0111**	**2.3508**	**0.0645**	**0.0001**	**13.3291**	**2.9758**

Numbers in bold represent the indicator families of each location

APPENDIX H: DETAILED INFORMATION ON ALL AMF TAXA

Table 32. Detailed taxonomy of all identified AMF

VT ID	VT size	Taxonomy
VTX00388	349148	gi|ESA02435|gb|FN263101| Glomeraceae Glomus Alguacil09b Glo G1
VTX00155	251637	gi|MOA02053|gb|HF567092| Glomeraceae Glomus sp.
VTX00105	228390	gi|MOA08129|gb|HG004469| Glomeraceae Glomus Torrecillas 13 Glo G3
VTX00193	211816	gi|MOA01193|gb|JF683559| Claroideoglomeraceae Claroideoglomus sp.
VTX00098	177840	gi|MOA03382|gb|HE578040| Glomeraceae Glomus sp.
VTX00342	171220	gi|MOA00008|gb|FN645968| Glomeraceae Glomus Glo G2b
VTX00355	58965	gi|MOA01195|gb|JF683561| Diversisporaceae Diversispora sp. T
VTX00444	54195	gi|MOA37268|gb|LT982355| Paraglomeraceae Paraglomus IH1
VTX00419	43745	gi|MOA00083|gb|GU353731| Glomeraceae Glomus LES19
VTX00308	42440	gi|ESA02692|gb|EU123462| Paraglomeraceae Paraglomus Glom 1B.13 T
VTX00100	34727	gi|MOA02715|gb|FR750209| Glomeraceae Glomus intraradices
VTX00354	24687	gi|MOA03543|gb|HE615058| Diversisporaceae Diversispora Torrecillas12b Div3
VTX00148	18374	gi|ESA01962|gb|DQ085215| Glomeraceae Glomus JP5
VTX00265	12895	gi|MOA02712|gb|FR773145| Glomeraceae Glomus coronatum
VTX00357	12306	gi|MOA01183|gb|HE576932| Claroideoglomeraceae Claroideoglomus Alguacil12b GLO G3
VTX00311	12235	gi|ESA02403|gb|FM876953| Glomeraceae Glomus Alguacil09a Glo unk5 T
VTX00395	11122	gi|MOA00707|gb|FR693586| Glomeraceae Glomus Glo G4
VTX00140	9780	gi|ESA01868|gb|AJ563896| Glomeraceae Glomus Wirsel OTU13 T
VTX00067	8763	gi|ESA02182|gb|AJ306438| Glomeraceae Glomus mosseae T
VTX00092	7185	gi|MOA01737|gb|HF566776| Glomeraceae Glomus sp.
VTX00024	4840	gi|ESA00828|gb|AY394664| Acaulosporaceae Acaulospora sp. T
VTX00166	4347	gi|MOA08386|gb|AB749512| Glomeraceae Glomus Goomaral13b Glo 10
VTX00156	3785	gi|MOA03009|gb|HE798975| Glomeraceae Glomus sp.

VTX00167	3512	gi|ESA01220|gb|EU350060| Glomeraceae Glomus Glo-F T
VTX00153	3445	gi|ESA01321|gb|DQ357089| Glomeraceae Glomus sp.
VTX00063	2916	gi|MOA08747|gb|KF386338| Glomeraceae Glomus sp.
VTX00312	2696	gi|MOA00017|gb|FN645993| Glomeraceae Glomus Glo G3
VTX00056	2546	gi|MOA02476|gb|GU238403| Claroideoglomeraceae Claroideoglomus Glo-B2
VTX00402	2393	gi|MOA08153|gb|HG004493| Claroideoglomeraceae Claroideoglomus Torrecillas 13 Cl3
VTX00005	2017	gi|MOA08580|gb|KC708346| Archaeosporaceae Archaeospora Desiro13a MIB 8531
VTX00377	1344	gi|MOA02776|gb|HE798742| Diversisporaceae Diversispora MO-D1
VTX00154	1235	gi|MOA03005|gb|HE798971| Glomeraceae Glomus sp.
VTX00295	1110	gi|MOA02537|gb|JN009218| Glomeraceae Glomus sp.
VTX00064	1031	gi|MOA00717|gb|FR693410| Glomeraceae Glomus Glo G5
VTX00004	851	gi|MOA00332|gb|GQ140623| Archaeosporaceae Archaeospora sp.
VTX00054	814	gi|ESA01188|gb|AJ315524| Diversisporaceae Diversispora sp. T
VTX00072	774	gi|MOA03202|gb|HE799168| Glomeraceae Glomus sp.
VTX00009	711	gi|MOA08609|gb|KC708375| Archaeosporaceae Archaeospora sp.
VTX00263	702	gi|ESA02176|gb|Y17650| Diversisporaceae Diversispora spurca T
VTX00411	693	gi|MOA00180|gb|GU353720| Glomeraceae Glomus LES15 T
VTX00356	681	gi|MOA08399|gb|AB749527| Diversisporaceae Diversispora Goomaral13b Div 1
VTX00304	441	gi|ESA02524|gb|FM875902| Glomeraceae Glomus sp. T
VTX00102	396	gi|ESA01513|gb|AY512359| Acaulosporaceae Acaulospora Acau16 T
VTX00008	349	gi|ESA01484|gb|EU573738| Archaeosporaceae Archaeospora Schechter08 Arch1 T
VTX00143	328	gi|ESA00688|gb|AJ309460| Glomeraceae Glomus Glo4
VTX00362	283	gi|MOA01791|gb|HF566830| Glomeraceae Glomus MO-G39
VTX00204	276	gi|ESA01253|gb|EU417636| Glomeraceae Glomus Kupea martinetugei symbiont
VTX00163	255	gi|ESA01920|gb|AJ563876| Glomeraceae Glomus Wirsel OTU8
VTX00030	166	gi|MOA02730|gb|HE798696| Acaulosporaceae Acaulospora MO-A9
VTX00310	140	gi|ESA02549|gb|FJ194510| Glomeraceae Glomus ORVIN GLO3D T
VTX00423	130	gi|MOA08519|gb|HE613471| Glomeraceae Glomus Alguacil14b Glo10 T

VTX00283	127	gi|ESA01948|gb|DQ396687| Ambisporaceae Ambispora PF27
VTX00338	113	gi|MOA00681|gb|FN869851| Archaeosporaceae Archaeospora Aca
VTX00372	101	gi|MOA01468|gb|HF566507| Glomeraceae Glomus MO-G49 T
VTX00049	101	gi|MOA02595|gb|JN009380| Gigasporaceae Scutellospora Liu2012b Phylo-33
VTX00108	96	gi|ESA02688|gb|EU123431| Glomeraceae Glomus Glom 1B.10
VTX00363	96	gi|MOA03384|gb|HE578046| Glomeraceae Glomus sp.
VTX00380	77	gi|MOA00620|gb|FN869704| Diversisporaceae Diversispora Div T
VTX00293	75	gi|ESA01941|gb|DQ396759| Glomeraceae Glomus PF20
VTX00359	73	gi|MOA02666|gb|JQ246065| Glomeraceae Glomus sp.
VTX00242	73	gi|ESA02201|gb|AJ301861| Ambisporaceae Ambispora leptoticha
VTX00393	67	gi|MOA02587|gb|JN009357| Glomeraceae Glomus Liu2012b Phylo-3
VTX00396	62	gi|ESA02440|gb|FN263106| Glomeraceae Glomus Alguacil09b Glo G4
VTX00112	57	gi|ESA01146|gb|DQ336482| Glomeraceae Glomus Kottke08-4 T
VTX00135	55	gi|MOA08049|gb|KF467269| Glomeraceae Glomus Shi14b Glo-9
VTX00186	42	gi|MOA01616|gb|HF566655| Glomeraceae Glomus sp.
VTX00060	41	gi|MOA01438|gb|HF566477| Diversisporaceae Diversispora sp.
VTX00185	31	gi|MOA08550|gb|KF612333| Glomeraceae Glomus Deepika15 Glo P21
VTX00315	30	gi|MOA02281|gb|HF567320| Glomeraceae Glomus sp.
VTX00418	23	gi|MOA00084|gb|GU353730| Glomeraceae Glomus LES19
VTX00187	22	gi|MOA03068|gb|HE799034| Glomeraceae Glomus sp.
VTX00398	18	gi|MOA02811|gb|HE798777| Glomeraceae Glomus MO-G41 T
VTX00301	17	gi|MOA08452|gb|AB698610| Glomeraceae Glomus Goomaral13a Glo4
VTX00390	16	gi|ESA02429|gb|FN263090| Glomeraceae Glomus Alguacil09b Glo G16 T
VTX00070	15	gi|MOA00370|gb|HM440269| Glomeraceae Glomus sp.
VTX00175	15	gi|ESA00522|gb|AM412105| Glomeraceae Glomus Glo8 T
VTX00364	14	gi|MOA01465|gb|HF566504| Glomeraceae Glomus MO-G48 T
VTX00361	14	gi|MOA02840|gb|HE798806| Glomeraceae Glomus MO-G57
VTX00165	13	gi|ESA01210|gb|EF154348| Glomeraceae Glomus sp.

VTX00403	13	gi|ESA01302|gb|AB178716| Glomeraceae Glomus PSAMG2
VTX00384	13	gi|MOA02842|gb|HE798808| Glomeraceae Glomus MO-G59 T
VTX00349	12	gi|MOA08546|gb|HE613489| Paraglomeraceae Paraglomus Alguacil14b Para2
VTX00280	12	gi|MOA03143|gb|HE799109| Glomeraceae Glomus sp.
VTX00435	12	gi|MOA17012|gb|LN623503| Paraglomeraceae Paraglomus MO-P4 T
VTX00069	12	gi|MOA03189|gb|HE799155| Glomeraceae Glomus sp.
VTX00307	11	gi|MOA08284|gb|KF049901| Glomeraceae Glomus sp.
VTX00326	9	gi|MOA00554|gb|EU340316| Glomeraceae Glomus NF23 T
VTX00160	9	gi|MOA03168|gb|HE799134| Glomeraceae Glomus sp.
VTX00248	8	gi|ESA02519|gb|FM875894| Glomeraceae Glomus sp.
VTX00237	8	gi|ESA02056|gb|AF202280| Claroideoglomeraceae Claroideoglomus sp. T
VTX00278	8	gi|MOA00060|gb|GU353691| Claroideoglomeraceae Claroideoglomus LES09
VTX00350	8	gi|MOA01125|gb|HE576814| Paraglomeraceae Paraglomus Alguacil12b PARA1 T
VTX00019	8	gi|ESA00209|gb|AJ496112| Acaulosporaceae Acaulospora MO-A2
VTX00234	7	gi|MOA00657|gb|FN869783| Glomeraceae Glomus Glo G5
VTX00399	7	gi|MOA02838|gb|HE798804| Glomeraceae Glomus MO-G56 T
VTX00347	6	gi|ESA01947|gb|DQ396738| Diversisporaceae Diversispora PF26
VTX00352	6	gi|MOA01119|gb|HE576802| Paraglomeraceae Paraglomus Alguacil12b ACA1 T
VTX00137	6	gi|ESA01871|gb|AJ563890| Glomeraceae Glomus Wirsel OTU14 T
VTX00130	5	gi|MOA07991|gb|JX999400| Glomeraceae Glomus Shi14a Phy-6
VTX00397	5	gi|MOA01449|gb|HF566488| Glomeraceae Glomus MO-G38
VTX00409	5	gi|MOA03555|gb|HE615074| Glomeraceae Glomus Torrecillas12b Glo G13 T
VTX00215	5	gi|MOA02818|gb|HE798784| Glomeraceae Glomus MO-G45
VTX00149	5	gi|MOA03391|gb|FM877497| Glomeraceae Glomus Sanchez-Castro12a GLO6
VTX00101	4	gi|MOA02664|gb|JQ246063| Glomeraceae Glomus sp.
VTX00415	4	gi|MOA01614|gb|HF566653| Glomeraceae Glomus sp.
VTX00344	4	gi|ESA02586|gb|DQ263980| Glomeraceae Glomus sp.
VTX00331	4	gi|MOA00072|gb|GU353949| Glomeraceae Glomus NES27 T

VTX00332	4	gi|MOA00066|gb|GU353947| Glomeraceae Glomus NES25 T
VTX00057	3	gi|MOA07976|gb|JX999424| Claroideoglomeraceae Claroideoglomus sp.
VTX00256	3	gi|MOA03133|gb|HE799099| Glomeraceae Glomus sp.
VTX00088	3	gi|TJA00014|gb|AB546417| Glomeraceae Glomus sp.
VTX00238	3	gi|ESA02145|gb|AJ276082| Paraglomeraceae Paraglomus occultum
VTX00028	3	gi|MOA08704|gb|KF386321| Acaulosporaceae Acaulospora sp.
VTX00065	2	gi|MOA08253|gb|KF049823| Glomeraceae Glomus sp.
VTX00089	2	gi|MOA02659|gb|JQ246058| Glomeraceae Glomus sp.
VTX00099	2	gi|ESA02218|gb|AF213462| Glomeraceae Glomus proliferum T
VTX00061	2	gi|MOA02786|gb|HE798752| Diversisporaceae Diversispora sp.
VTX00327	2	gi|MOA03177|gb|HE799143| Glomeraceae Glomus sp.
VTX00328	2	gi|MOA00724|gb|JF414178| Acaulosporaceae Acaulospora sp. T
VTX00365	2	gi|MOA01312|gb|HM215930| Glomeraceae Glomus sp.
VTX00219	2	gi|MOA00161|gb|GU353435| Glomeraceae Glomus LER02
VTX00245	2	gi|MOA00729|gb|JF414183| Archaeosporaceae Archaeospora sp.
VTX00077	2	gi|MOA03225|gb|HE799191| Glomeraceae Glomus sp.
VTX00450	2	gi|MOA28042|gb|LT831928| Archaeosporaceae Archaeospora sp.
VTX00323	2	gi|MOA00537|gb|GU183691| Glomeraceae Glomus Glo-C T
VTX00177	2	gi|MOA03061|gb|HE799027| Glomeraceae Glomus sp.
VTX00447	2	gi|MOA35125|gb|LT723028| Glomeraceae Glomus sp.