

CONTRIBUTIONS OF PLANT MICROBE INTERACTIONS TO ENHANCE
DROUGHT RESISTANCE AGAINST FOREST FIRES

by

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Integrated B.S. and M.S. Program in Teaching Chemistry, Boğaziçi University, 2016

Submitted to the Institute of Environmental Sciences in partial fulfillment of

the requirements for the degree of

Master of Science

in

Environmental Sciences

Boğaziçi University

2019

ACKNOWLEDGEMENTS

Firstly, I would like to express my deep and sincere gratitude to my thesis advisor Assist. Prof. Dr. M. Ali Khalvati and my co-advisor Prof. Dr. M. Levent Kurnaz for allowing me to do research and providing invaluable guidance throughout this study. It has been a great pleasure to study with them.

I would like to thank Prof. Dr. Melek Türker Saçan not only for her laboratory support in my greenhouse experiment but also, for guidance and her advises during my study

I thank my colleague MSc. Mehmet Meriç Tunalı for his support during the greenhouse study. I would also thank MSc. H. Aygün Karaçay and MSc. Ece Sayın for their sincere friendship.

I thank my İKLİMBU family, MSc. Kamil Çöllü, MSc. Aytaç Paçal, MSc. Nazan An and, MSc. M. Tufan Turp for their support and friendship during my thesis study.

I am extremely grateful to my dearest, closest friend MSc. Zeynep Yılmaz for constant support in all stages of my master's degree. I am also thankful to my best friends MSc. Özlem Şengül and MSc. Ezgi Sunar for their friendship throughout my master's degree.

Finally, I extend my deepest thanks to my dearest family, my mother Emine Calda, my sister Duygu Calda and my brother Murat Calda who have always supported and been there for me through my life. My love for them is indescribable.

ABSTRACT

CONTRIBUTIONS OF PLANT MICROBE INTERACTIONS TO ENHANCE DROUGHT RESISTANCE AGAINST FOREST FIRES

Forest fires are natural in the Mediterranean ecosystems. However, in the last decade, the number of wildfires has significantly increased in the Mediterranean basin along with climate change. Therefore, forecasts of this region by using fire indices are crucial to take necessary precautions. In the present study, the projected changes for the period 2070 - 2099 concerning the control period 1971 - 2000 were used to estimate forest fire risk by the Canadian Fire Weather Index (FWI) and the greenhouse experiment was conducted to investigate the contribution of plant-microbe interaction against wildfires. RCP4.5 and RCP8.5 emission scenario (IPCC) output of MPI-ESM-MR and HadGEM2-ES dynamically downscaled to 50 km for the CORDEX-MENA domain with the use of the RegCM4 were utilized. ERA-Interim observational data from ECMWF covering the period 1980-2012 were also used to test the performances of models. The output of MPI-ESM-MR gave more similar fire risk prediction with the reforecast of observational data (ERA-Interim). Thus, the MPI-ESM-MR model could be more suitable to estimate fire risk by FWI. According to future projection, forest fire risk will significantly increase throughout the region for the last 30 years of this century. In the greenhouse experiment, interaction between arbuscular mycorrhizal fungi (AMF) and host plant was evaluated under high temperature and water stress by using soil collected from the Muğla forest area and the mining area in Kütahya, *Pinus nigra* (Turkish pine) sapling, *Quercus* (oak) sapling, and, *Glomus mosseae* since AMF significantly affect the functioning of forest ecosystems by improving the ability of water and nutrient uptake from soil in their host plants. The consequence of the study shows that AMF enhances plant growth, symbiotic relations, and drought tolerance in the host plants.

ÖZET

ORMAN YANGINLARINA KARŞI BİTKİ-MİKROPETKİLEŞİMLERİNİN KURAKLIK DİRENCİNİ ARTIRMADAKİ KATKISI

Akdeniz ekosistemlerinde orman yangınları normaldir. Bununla birlikte, son on yılda, Akdeniz havzasında iklim değişikliği ile orman yangınlarının sayısı önemli ölçüde artmıştır. Bu nedenle, bu bölgede yangın indisleri kullanılarak yapılan öngörüler gerekli önlemleri almak için çok önemlidir. Bu çalışmada, 1971- 2000 kontrol periyodu temel alınarak 2070- 2099 dönemi için Kanada Orman Yangın Hava İndisi (FWI) kullanılarak öngörülen değişiklikler orman yangını riskini tahmin etmek için kullanılmıştır; Sera çalışması, orman yangınlarına karşı bitki-mikrop etkileşimlerinin kuraklık direncini artırmadaki katkısını araştırmak amacıyla yapılmıştır. RCP4.5 ve RCP8.5 senaryo setleri alt ölçeklendirme yöntemi ile 50 km çözünürlükte koşulmuş MPI-ESM-MR ve HadGEM2-ES küresel iklim modelleri kullanılmıştır. Ayrıca, ECMWF'nin 1980-2012 dönemi kapsayan ERA-Interim gözlem verisi modellerin performansını test etmek için kullanılmıştır. Geçmiş yangın riski öngörüsünde MPI-ESM-MR modeli gözlem verisi (ERA-Interim) ile çok benzer sonuçlar vermiştir. Bu yüzden, FWI ile yangın riskini tahmin etmede MPI-ESM-MR modeli bu bölge için daha uygun olabilir. Gelecek projeksiyonuna göre orman yangını riski bu yüzyılın son 30 yılında bölge genelinde önemli ölçüde artacaktır. Sera çalışmasında, yüksek sıcaklık ve su stresi altında arbüsküler mikorizal fungus (AMF) ve konuk bitki arasındaki etkileşim, Muğla orman bölgesinden ve Kütahya maden bölgesinden alınan topraklar, *Pinus nigra* (Türk çamı) fidanı, *Quercus* (meşe) fidanı ve *Glomus mosseae* (AMF) kullanılarak değerlendirilmiştir. Çünkü AMF, konuk bitkilerin topraktan su ve besin alımını artırarak orman ekosistemlerinin işleyişini önemli ölçüde etkilemektedir. Çalışmanın sonucu, AMF' nin konuk bitkilerin büyümesini, simbiyotik ilişkilerini ve kuraklık toleransını arttırdığını göstermektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Abbreviation	Explanation
AM	Activity meter
AMF	Arbuscular mycorrhizal fungi
CFCs	Chlorofluorocarbons
CORDEX	Coordinated Regional Climate Downscaling Experiment
EC	Electrical Conductivity
ECMWF	The European Centre for Medium-Range Weather Forecasts
EFFIS	The European Forest Fire Information System
ERA-Interim	A global atmospheric reanalysis
FWI	The Canadian Fire Weather Index
GCMs	General Circulation Models
GHG	Greenhouse Gas
GRSP	Glomalin-Related Soil Protein
HadGEM2-ES	Hadley Global Environment Model 2 - Earth System
IPCC	the Intergovernmental Panel on Climate Change
MENA	Middle East and North Africa
MPI-ESM	The traditional Max Planck Institute Earth system model
RCD	Regional Climate Downscaling
RCPs	The Representative Concentration Pathways
RegCM4	Regional Climate Model version 4
TGRSP	Total Glomalin Related Soil Protein

1. INTRODUCTION

Wildland fires have a more harmful effect on trees and plant cover than parasites, insects, harsh weather conditions as well as similar natural disasters (Tatlı and Türkeş, 2014). In the regions with the Mediterranean climate, the temperature increases with climate change and this leads to rising the number of wildfires (Westerling et al., 2006; Turco et al., 2014). Studies about wildfires indicate that these fires are related not only to temperature increase but also to atmospheric instability and dryness (Jenkins, 2002, 2004; Choi et al., 2006; Winkler et al., 2007; Peace et al., 2012; Barbera et al., 2014). Besides, climate change leads to an increase in temperatures, drought, and constant temperature fluctuations. Thus, longer summer droughts and exacerbation of droughts in other seasons give rise to the risk of potential forest fires and burning areas in the region of the Mediterranean basin as well as in some areas of the Middle East and North Africa (MENA) (Tatlı and Türkeş, 2014).

Wildfires are a crucial part of Mediterranean ecosystems. In the last century, in the Western Mediterranean Region (Valencia Region), urbanization that causes an increase in fuel quantity and continuity (vegetation and fuel increase after the abandonment of farms) leads to an increase the number of wildfires since climatic conditions have a low impact on forest fires before the 1970s. However, the climate starts to have a significant effect on forest fires after the 1970s. Dry weather conditions are an important factor in the emergence of fires in recent years and this situation can be seen more frequently due to climate change (Pausas et al., 2012).

In many countries of Mediterranean Basin (and Turkey), especially in western and southern regions, wildland fires are at the top of the factors that threaten forests since the summers are very hot and arid, the winters are warm and rainy in the Mediterranean climate zone (Türkeş and Altan, 2012; Erkan, 2006; Altan, 2011; Altan et al., 2011). In Turkey, Muğla is one of the riskiest areas in terms of forest fires due to arid climate conditions. The high number of forest fires occurred in Muğla during the period between the months of July and September (Türkeş and Altan, 2012).

Additionally, drought and hot weather conditions are not enough to start a forest fire. Fire begins with combining of oxygen, heat, and fuel (grass or fine flammable materials on the surface, often referred to as flammable material on the surface) in a certain way since this situation leads to fuel ignition and combustion (Tatlı and Türkeş, 2014; Johnson and Miyanishi, 2001). Besides, a high amount of moisture serves stable abundant vegetation growth as well as provides a barrier to wildland fire growth until conditions occur that decrease moisture content below critical lower bound

(Abatzoglou and Kolden, 2013). Thus, the moisture content of soil and fuel masses have a significant influence on both combustibility and the wildland fire duration (Blackwell et al., 1992; Vallette et al., 1994; Dimitrakopoulos et al., 2010). Ultimately, moisture, fuel type, spatial continuity are crucial points to determine fire regime (Flannigan et al., 2000).

Climate models provide an estimation of potential wildland fires. To increase the number of wildfires leads to the need for understanding the relation between fire and weather to improve fire threat estimations (Raffault et al., 2016). The relationship between drought and forest fires is evaluated by many countries using different indices. Some of fire indices are widely used to predict potential forest fire risks are the Canadian Fire Weather Index (FWI), the Haines Index (HI), the Keetch-Byram Drought Index (KBDI), the McArthur Mark 5 (Mk5) Forest and Mark 4 (Mk4) Grassland Fire Danger Index, the Fosberg Fire Weather Index, and the Nesterov Index (Sharples et al., 2009a; Sharples et al., 2009b; Altan, 2011; Altan et al., 2011; Ganatsas et al., 2011; Perez et al., 2017). The Canadian Fire Weather Index (FWI) is one of the most preferred fire indices that presents a reliable forecast of extreme fire seasons (Bedia et al., 2018). Also, FWI is used as the official index for fire danger predictions issued by the European Forest Fire Information System (EFFIS, 2019). It could be used for forest fire management in MENA region.

Taking precautions about forest fires is crucial because wildfires bring about growing environmental and ecological problems. Arbuscular mycorrhizal fungi (AMF), for example, could be used to reduce the number of probable forest fires. Arbuscular mycorrhizal fungi (AMF) are the most common soil microorganisms in natural and agricultural soils. It provides their host plants to enhance both nutrient, water and metal uptake (He et al., 2014). Additionally, the soil microbial contributions that create symbiotic associations with host plants enhance the ability of water uptake from the soil as well as decrease drought stress in their host plants (Khalvati et al. 2005; Ruth et al. 2011). In this way, wildfires could slow down or be brought under control handily in drylands due to the relation between forest fire and fuel moisture.

1.1. Aim of the study

In this study, the projected changes for the period 2070 - 2099 (long term) in common climatic parameters of precipitation (mm), temperature (°C), relative humidity (%) and wind speed (km/h) concerning the control period 1971 - 2000 are used to investigate forest fire risk by FWI packages in R programming language (fireDanger). The outputs of MPI-ESM-MR and HadGEM2-ES are dynamically downscaled to 50 km for the CORDEX-MENA domain with the use of RegCM4. The projections are realized according to the RCP4.5 and the RCP8.5 concentration scenarios of the Intergovernmental Panel on Climate Change (IPCC). Furthermore, reanalysis data from ECMWF Era-Interim covering the period 1980-2012 are used to analyze forest fire risk by FWI.

Moreover, wildfires are related to atmospheric instability and dryness, as well as changes in the moisture content of different soil layers in the forest floor and change in fuel moisture content. Therefore, in the greenhouse experiment, plant-microbe (AMF) interaction are investigated to increase drought resistance against fires. In this experiment, soil moisture rate is measured by using soil taken from the Muğla forest area and a mine tailing area in Kütahya, *Pinus nigra* (Turkish pine) sapling, *Quercus* (oak) sapling and mycorrhiza (AMF).

All in all, the purposes of the present study are to provide potential fire behavior by FWI in the MENA region and to indicate AMF could be used as a precaution to prevent or to slow down forest fires in arid regions.

2. THEORETICAL BACKGROUND

2.1. Climate Change & Forest Fire

Climate generally defines the combination of the average characteristics of all-weather conditions observed for a long period of time (average 30 years) anywhere on the earth (Türkeş, 2010; WMO, 2019).

Climate change is a long-term and slow-growing changes in climate conditions that have large-scale and significant local effects. In the past, the geologic record demonstrates that serious changes in climate have taken place. These changes are called natural climate changes due to the occurrence in the absence of humans. After the industrial revolution, human activities like burning fossil fuels, land-use changes, deforestation, and industrial processes cause the increase of long-lived greenhouse gases including carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and chlorofluorocarbons (CFCs). Therefore, today, climate change is defined by considering human activities that increase greenhouse gas accumulations. For instance, the United Nations Framework Convention on Climate Change defines climate change as a change in climate as a result of human effects that directly or indirectly disrupt the composition of the global atmosphere, in addition to the natural climate change observed in a comparable time period. (Hartmann, 1994; Türkeş, 2010).

Many studies have addressed the relation between fire and weather by drawing attention to the impact of climate change and increasing temperatures (Pausas and Vallejo, 1999; Pausas, 2004; McCaw et al., 2007; Trouet et al., 2009; Urbietta et al., 2015). Urbietta et al. (2015) show that the effect of weather conditions on fires in two Mediterranean-type regions: five southern countries of the European Union (EUMED); the Pacific western coast of the USA (California and Oregon, PWUSA) by analyzing fire activity and climatic conditions during fire season (between March and August) for the 1985–2011 period. The study shows that climate has a significant impact on fire activity. Furthermore, several studies show that in the future under extremely hot weather conditions, fire seasons will prolong, and wildfires will increase (Gillet et al., 2004; Flannigan et al., 2006).

Wildfires are an integral component of Mediterranean ecosystems. Extreme temperature events in summers are a crucial factor to increase in the number of wildland fires. The relationship between forest fires and the climate was indicated by researching temperature and precipitation data from 350

meteorological stations and the number of fires between 1950 and 2000 in the Eastern Iberian Peninsula. The result of the study provides that changes in wildland fire frequency in these ecosystems are intimately linked to climate change (Pausas, 2004). Additionally, in some regions with the Mediterranean climate, like California, low humidity and extreme weather conditions raised by human-caused climate change are increasing the number of wildfires (Westerling et al., 2004; Schweizer et al., 2019).

The study carried by Abatzoglou et al. (2019) indicates that in the Anthropocene, rising fire weather due to climate change will continue to shape global forest fire activity. Additionally, weather and fuel aridity have a strong relationship with massive burned areas. The result of the study shows that potential fire risk and burned areas in Mediterranean Europe according to model estimation will increase with global warming. Therefore, adaptation efforts for wildfires will be required to restrain the adverse effects of fire.

2.2. Mediterranean Basin Climate

Köppen-Geiger climate classification that is based on seasonal temperature and precipitation patterns is mostly used for climate classification. The classification includes five main classes: A (tropical), B (dry), C (temperate), D (continental), and E (polar) and 30 sub-types (Figure-2.1) (Türkeş, 2010; Tatlı and Türkeş, 2014; Türkeş, 2016; Rubel et al., 2017; Beck et al., 2018). The Mediterranean basin where forest fires are frequently observed has temperate climates (Group C) in the Köppen-Geiger climate classification. Moist mid-latitude (temperate) is seen in the regions within the range of 25 - 60° latitudes. These areas include the Mediterranean basin. According to the Köppen-Geiger climate classification, the Mediterranean basin is in the Cs group, is generally mild and rainy in winters, and has a hot and arid climate type in summers. The Mediterranean climate is divided into two sub-groups: Csa (summers are very hot and dry) and Csb (summers are hot and dry). Places where these climates are seen; France, Italy, Greece, Turkey, Portugal, Spain, North Africa (Morocco, Tunisia, and Algeria), are Lebanon and Israel. The Mediterranean climate is also seen in northern Iran, California, Chile, Australia, New Zealand, and South Africa.

The Mediterranean basin also includes arid climates (Group B) and moist terrestrial climates (D group). Arid climates (group B) are the climates where annual evaporation is much higher than annual precipitation, so there is a continuous water shortage. Desert (BW) and Step (BS) climate are divided into two sub-groups. Steppe climate is predominantly in the Iberian and Anatolian peninsula, in the

vicinity of the Caspian Sea and in the Sahel (on the southern border of the Sahara) in North Africa. In the Mediterranean basin where the desert climate (BW) is seen as dominant, North Africa, the Middle East, and the Arabian Peninsula are the regions. The humid cold middle latitudes (D group) are observed in the Caucasus, Central Anatolia, and Eastern Anatolia regions in the Mediterranean basin. It is commonly called humid and climates with severe winters (Türkeş, 2010; Tatlı and Türkeş, 2014; Türkeş, 2016)

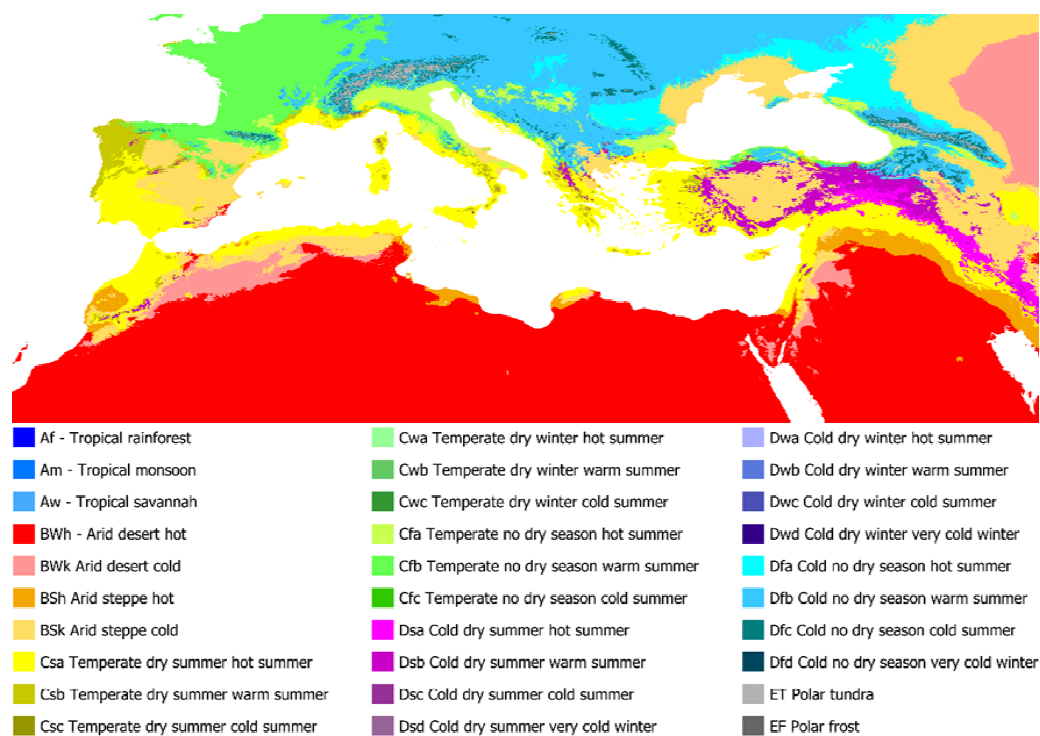


Figure 2.1. Köppen-Geiger climate classification (Beck et al., 2018)

2.3. Wildfire & Fuel Types

Fire scientists, in general, define three types of fuels, which are aerial fuel with all green and dead materials in the upper forest canopy, surface fuel with grass or thin combustible materials lying on the forest ground and finally ground fuel with all ignitable materials under the surface such as roots, duff. Aerial fuel usually includes higher moisture and less fuel load content than surface fuel (Figure-2.2).

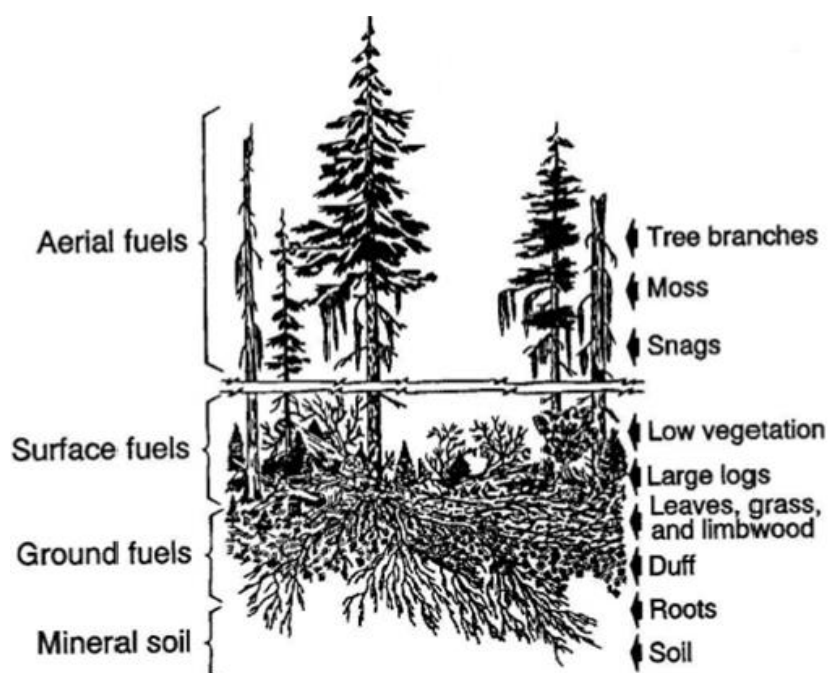


Figure 2.2. Fuel components are classified as ground, surface and aerial (Salis, 2008)

There are three types of wildland fires as regards to fuel types where fire spreads; ground, surface, and crown fires. A ground fire adversely affects ground fuels and it leads to crucial damage to trees and shrubs. A surface fire spreads on the surface fuel layer including needles or leaves, duff, grass, small dead wood, downed logs, stumps, large limbs, and low shrubs. Ultimately, a crown fire burns the elevated forest canopy. Moreover, controls of ground and surface fires are easier than crown fires control. Additionally, the consequences of crown fires are serious and permanent due to the loss of some tree species (Van Wagner, 1977; Rothermel, 1983; Ferraz et al., 2009).

2.4. The Canadian Fire Weather Index (FWI)

The Canadian Fire Weather Index (FWI) system firstly published in 1970 by using several results of fire researches in the Canadian Forestry Service (Van Wagner, 1987). It was improved for Canadian forests but today, the FWI system is most widely used in other countries such as Mexico, south-east Asia, Florida, Argentina. Additionally, several studies indicate that the Canadian Fire Weather Index is suitable for the forecasting forest fire risk for the Mediterranean region (Moriondo et al, 2006; Viegas et al. 2001, Aguado et al. 2003; Carvalho et al., 2008; Bedia et al., 2017).

Both climate and weather conditions have an important role to decide fire regime. Drought weather condition affects fuel moisture and lightning ignitions and this situation leads to increasing

wildfires. The Canadian Fire Weather Index (FWI) is the most preferred index to estimate wildland fire disasters (Carvalho et al., 2008) since the FWI system associate weather information with fuel moisture and fire hazard indices. Additionally, this system is adapted to different forest types (Wotton, 2009; Chelli et al., 2015). Some research done in Mediterranean regions indicates that there is a strong relationship between the weather and the FWI system. For this reason, the Canadian Fire Weather Index forecast accomplished fire hazard for Mediterranean countries (Carvalho et al., 2008; Chelli et al., 2015).

Wildland fires start to be a significant issue in the Mediterranean region (Chelli et al., 2015) since, in this region, Mediterranean climate is seen which winters are mild and rainy, and summers are hot and arid (Türkeş, 2010, Climatology and Meteorology). Besides, some studies show that in regions where the Mediterranean climate is observed, the temperature increases with the climate change and this increment leads to a rise in the number of forest fires (Schröter et al., 2005; Westerling et al., 2006; Bedia et al., 2017). Furthermore, the Aegean and Mediterranean coastal regions of Turkey are under high fire risk owing to the plant cover and climate conditions. Especially, the high number of wildfires is seen in Çanakkale, İzmir and Muğla regions. Thus, these regions have the highest forest fire risk in Turkey. The result of research in these three regions demonstrates that the Canadian Fire Weather Index system is statistically significant in clarifying forest fire risk and burnt area (Ertuğrul and Erol, 2016).

2.5. Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) are widespread symbiotic organisms in the phylum Glomeromycota can have a wide variety of impacts on the host plant growth. 80% of present vascular plant families include AMF. (Schüssler et al. 2001; Auge, 2004; Rillig, 2004). AMF enhance the physical quality of plant rhizosphere and preserve plant against soil-borne pathogens (Hamel and Strullu, 2006). AMF symbioses rest upon the bilateral transfer of organic C from the plant and soil nutrients such as phosphorus (P), nitrogen (N) and zinc from the fungi (Jones et al., 2004; Lu et al., 2015).

Khalvati and colleagues (2005) investigated AMF can increase water and nutrient uptake from the soil. According to study, shoot and root biomasses of AMF plants were larger than non-AMF plants' biomasses under water stress since mycorrhizal contribution positively affects plant growth. Also, root water uptake was higher in mycorrhizal plants as compared to non-mycorrhizal plants. 4%

of water was transferred from the fungal hyphae to the host plant under drought conditions. The study shows that AMF has an important role in reducing drought stress in their host plants.

Plant-microbe interactions in the rhizosphere have serious effects on both plant health and soil fertility. AMF is the most crucial microbial organism for the greater part of plants due to maintaining soil fertility (Jeffries et al., 2002). Moreover, AMF improves plant tolerance to severe environmental stress such as salinity. AMF provides plants to increase the activity of antioxidant enzymes so arbuscular mycorrhizal plants overcome the reactive oxygen species produced by salinity. Thereby, arbuscular mycorrhizal plants have enough water status in their tissues (Al-Karaki, 2000; Porcel et al., 2012).

2.6. Inoculation of Arbuscular Mycorrhizal Fungi to Tree

Arbuscular mycorrhizal fungi (AMF) are ubiquitous endophytic fungi and have a crucial role in vegetation growth in ecosystems. They form symbiotic relations with the root of the greater part of plants (Lu et al., 2015). Studies about AMF species such as *Glomus mosseae* and *Glomus intraradices* inoculated seedlings indicate not only increasing plant growth but also improving resistance to water stress (Meddad-Hamza et al., 2010; Zhang et al., 2013). Moreover, the study made under greenhouse conditions shows that inoculation of *Glomus mosseae* and *Glomus intraradices* to *C. Glauca* (commonly known as the swamp oak) seedlings increased both nutrient uptake and the drought tolerance of seedlings. The result of the study represents mycorrhizal seedlings had bigger height, leaf area, and biomass than non-mycorrhizal seedlings have under water stress conditions (Zhang et al., 2013).

Underwater deficiency conditions, *Acacia senegal L.* seedlings are inoculated with *Glomus intraradices*, *G. fasciculatum* and *G. mosseae* that are different species of arbuscular mycorrhizal fungi (AMF) in a greenhouse study. Three water levels that are field capacity (FC), moderate water deficiency (50% FC) and severe water deficiency (25% FC) are carried out to the seedlings. The plants are grown for three months and in a consequence of study indicate that under drought conditions, AMF improves plant growth and symbiotic relations. Furthermore, the results provide that symbiosis enhance plant tolerance to water drought conditions (Ndiaye et al., 2011).

2.7. Glomalin Related Soil Protein (GRSP)

Glomalin, a glycoprotein, is produced by the hyphae of arbuscular mycorrhizal fungi (AMF), which enhances soil physical quality. It has a crucial role in the formation and stabilization of soil aggregates (Wright and Upadhyaya 1998, Rillig 2004). Glomalin is capable of sequestering significant amounts of C and N, so it is a serious resource of organic matter in the soil (Hamel and Strullu, 2006, Treseder and Turner, 2007). Rillig (2004) indicated that glomalin related soil protein (GRSP) concentrations in soil are strongly and positively correlated with aggregate water stability. Thus, AMF infections and glomalin could be advantageous to control desertification and soil degradation (Zhang et al., 2017).

Rillig and colleagues (2003) show the distribution of glomalin in soil horizons. Glomalin was found in A, B and C horizons and the total concentration of glomalin decreased in the A, B and C horizons respectively. According to the study, after 413 days of incubation of the forest and agricultural soils in A horizon, 50% of glomalin concentration was persistent. Additionally, glomalin stocks of forest soils were higher than agricultural soils. The consequences of the study called attention to the positive correlation of glomalin with soil organic C and N. Glomalin would be beneficial in controlling land-use change impacts on deciduous forest soils.

Lozano and colleagues (2016) demonstrated that the sensitivity of glomalin related soil protein (GRSP) stocks was clear to wildfires. According to study, GRSP in soil started to significantly decrease at 250 and 300 °C and this related to spatial distribution of vegetation since the range of maximum temperatures are between 300 - 700 °C in shrublands, which is higher than the range of maximum temperature in tree forest (200 - 300 °C). It leads to the differences in GRSP content in the burned area after the fire. The result of the study indicates that GRSP and soil organic carbon (SOC) provide useful information about fire intensity, so GRSP can be used as an indicator of soil health and recovery after wildfires.

3. METHODOLOGY

3.1. The Canadian Fire Weather Index (FWI) Calculation

Firstly, the Canadian Fire Weather Index (FWI) is one of the forest fire indices widely used in forest fire studies as well as is successful in predicting possible forest fires (Beverly and Wotton., 2007; Dimitrakopoulos et al., 2011; Chelli et al., 2015; Satir et al., 2016). The Canadian Fire Weather Index (FWI) considers moisture changes in different soil layers on the forest floor. The index calculates possible forest fires by evaluating fuel moisture values and current weather conditions together. The Canadian Fire Weather Index consists of six components. Calculation of the FWI System components is made by using noon temperature, relative humidity, wind speed, and daily total precipitation. The first three components are FFMC, DMC, and DC moisture codes (Wagner, 1987; Canadian Forestry Service, 2008):

- ***Fine Fuel Moisture Code (FFMC)*** shows the moisture content of dry grass and other combustible materials with an area of 0-1.2 cm dept (litter layer) (0.25 kg / m² load) on the forest floor. FFMC that is a numeric rating of moisture content is developed by the Tracer Index. FFMC permits conversions from code fine fuel moisture content (m) in percent. The real moisture content of pine litter ranges up to about 250 percent. The potential code scale length is 101. FFMC is fine fuel scales describes by the following equation:

$$FFMC = \frac{59.5(250-m)}{147.2+m} \quad (1)$$

- ***The Duff Moisture Code (DMC)*** shows the moisture content of decomposing organic materials at a depth of 1.2 to 7 cm (5 kg / m² load) on the forest floor. DMC is calculated by using moisture content (m) with the following equation:

$$DMC = 244.72 - 43.43 \ln(m - 20) \quad (2)$$

- ***Drought Code (DC)*** shows the organic moisture content in the forest floor at a depth of 7+ cm (25kg / m² load). Current drought code (D) equation is identified by using drying phase equations.

Drying Phase:

Potential evapotranspiration (V) is figured by an empirical equation depending noon temperature (T) and the seasonal daylength adjustment by month (L_f):

$$V = 0.36(T + 2.8) + L_f \quad (3)$$

The present day's DC is calculated utilizing initial drought code (D_o) and evapotranspiration (V):

$$D = D_o + 0.5V \quad (4)$$

Initial Spread Index (ISI) and Build-Up Index (BUI) were obtained by adding wind speed values to FFMC, DMC and DC humidity codes. Fire Weather Index was formed by the combination of previous forest fire indexes (Initial Spread Index (ISI) and Build-Up Index (BUI)) (Figure 1) (Van Wagner, 1987; Canadian Forestry Service, 2008; Lawson and Armitage, 2008).

-Initial Spread Index (ISI):

The equation of ISI is found by using wind and FFM functions, which represent the rate of potential fire spread. Wind function is used to compare airport winds with winds at fire weather stations since lots of airport locations show similarity with wind speed ratios compares to free-air winds as open fields compared to an opening surrounded by pine stands. Wind effect is a simple exponential, as shown by the following equation and W is the 10-meter open-wind speed in kilometers per hour (Lawson and Armitage, 2008):

$$\text{Wind function: } f(W) = e^{0.0503 W} \quad (5)$$

Function for fine fuel moisture (FFM) is depend on an analysis of the result of FFM in former fire danger system. FFM function, $f(F)$, includes fine fuel moisture content (m) in percent, as shown by following equation:

$$\text{FFM function: } f(F) = e^{-0.1386m} \left(1 + \frac{m^{5.31}}{4.93 \times 10^7}\right) \quad (6)$$

ISI is found by using wind and FFM functions, together with reference constant 0.208, as shown following equation:

$$ISI = 0.208f(W)f(F) \quad (7)$$

- Build-Up Index (BUI):

BUI is a combination of the DMC and the ISI and it indicates existing fuel for combustion:

$$BUI = \frac{0.8(DMC)(DC)}{(DMC+0.4DC)} \quad (8)$$

-Forest Weather Index (FWI):

Finally, Forest Weather Index (FWI) that describes potential fire intensity is figured dependent on Initial Spread Index (ISI) and Build-Up Index (BUI) by following equation:

$$BUI < 80 \quad f(D) = 0.626BUI^{0.809} + 2 \quad (9)$$

$$BUI > 80 \quad f(D) = \frac{1000}{25 + 108,64 e^{-0.023BUI}} \quad (10)$$

$$\ln(FWI) = 2.72(0.434 \ln(0.1ISI f(D)))^{0.647} \quad (11)$$

If $(0.1 ISI f(D)) < 1$ then $FWI = (0.1 ISI f(D))$

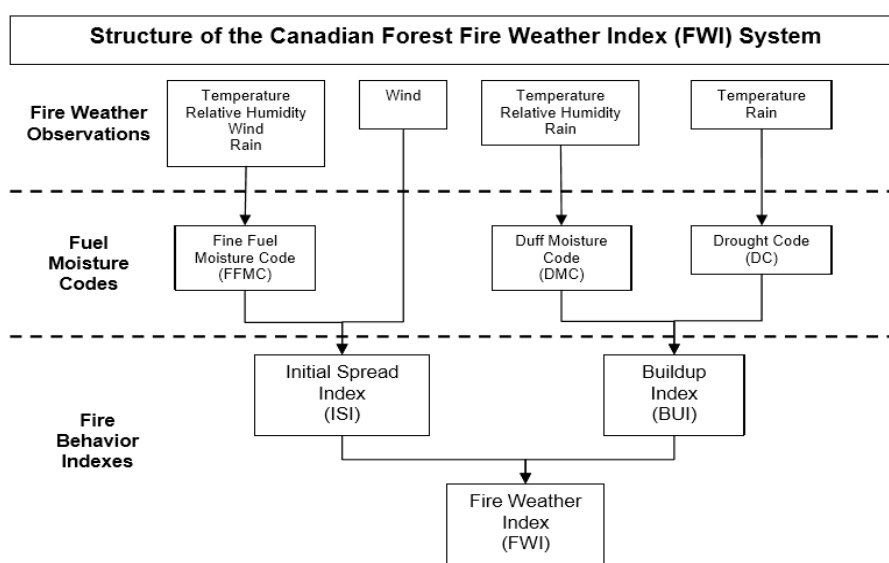


Figure 3.1. FWI System (de Groot, 1987)

Fire danger risk classification of the FWI defines in 6 classes: very low, low, medium, high, very high, and extreme and used in Canada (Van Wagner, 1987). The range of values is as in Table 3.1.

Table 3.1. FWI Classifications

Classification	FWI Values
Extreme	30+
Very high	17 - 29
High	9 - 16
Moderate	5 - 8
Low	2 - 4
Very low	0 - 1

The FWI classes are redefined with simple thresholding to assess the fire danger level in a conformed way Europe by the European Forest Fire Information System (EFFIS) and fire danger is ranged in 6 classes (very low, low, medium, high, very high and extreme) (EFFIS, 2019). In this study, EFFIS FWI classification was used to assess fire danger risk since it is more suitable for the Mediterranean region.

Table 3.2. EFFIS FWI Classifications

Classification	FWI Values
Extreme	≥ 50.0
Very high	38.0 – 50.0
High	21.3 – 38.0
Moderate	11.2 – 21.3
Low	5.2 – 11.2
Very low	< 5.2

3.1.1. Global Circulation Models (GCMs)

General Circulation Models (GCMs) are numerical models showing physical processes in the atmosphere, ocean, cryosphere, and land surface. GCMs simulate the response of the global climate system to rising greenhouse gas concentrations so they are presently the most advanced tools. GCMs utilize a three-dimensional grid over the globe, generally having a horizontal resolution of between 250 and 600 km, 10 to 20 vertical layers in the atmosphere, and sometimes 30 layers in the oceans. Spatial resolutions are quite coarse thus patterns created by GCMs are a poor representation (The IPCC Data Distribution Centre, 2015; Houghton, 2009).

3.1.2. MPI-ESM-MR Global Climate Model, ERA-Interim & HadGEM2-ES

The traditional Max Planck Institute Earth system model (MPI-ESM) compose of the component models ECHAM6 for the atmosphere, MPIOM for the ocean and JSBACH for the land and the vegetation. This improved version of the climate model permits studying feedbacks of climate change on the carbon cycle itself and is available for use by the scientific community (Giorgetta et al., 2013; New Earth system model of Max Planck Institute for Meteorology, MPI-ESM).

ERA-Interim is a global reanalysis that is generated by the European Centre for Medium-Range Weather Forecasts (ECMWF). ERA-Interim data provides a multivariate, spatially whole and consistent record of spherical atmospheric circulation (Dee et al., 2011).

HadGEM2-ES is a unified Earth System Model that is used for operational weather forecasting and climate research by the Met Office Hadley Centre for the Coupled Model Intercomparison Project (CMIP5). This model contains a unified atmosphere-ocean configuration and Earth system components which includes dynamic vegetation, ocean biology, and atmospheric chemistry. (Collins et al., 2011).

In the present study, the outputs of the Earth System model MPI-ESM-MR of the Max Planck Institute for Meteorology (MPI-M), ERA-Interim and HadGEM2-ES were used for assessing fire danger risk.

3.1.3. RegCM4 and RCP Scenarios

The Regional Climate Model (RegCM) was originally developed at the National Center for Atmospheric Research (NCAR) in 1989 and then is sustained in the Earth System Physics (ESP) section of the International Centre for Theoretical Physics (ICTP). RegCM4 is the latest version of the model and is now entirely promoted by the ESP and made applicable for public use. Former versions of the model are no longer available. The last version of the model involves new land surface, planetary boundary layer, and air-sea flux schemes, mixed convection and tropical band configuration, modifications to the pre-existing radiative transfer and boundary layer schemes. Additionally, RegCM4 is flexible, portable and easy to use. Also, it can be used for any region of the world with a grid spacing of up to 10 km and process studies to paleoclimate and future climate simulation. (ICTP, 2010; Giorgi et al., 2012).

The Representative Concentration Pathways (RCPs) are used for making projections based on anthropogenic greenhouse gas (GHG) emissions to explore possible future climate. The RCPs identify four different 21st-century pathways of GHG emissions and atmospheric concentrations, air pollutant emissions and land-use. The RCPs contain a stringent mitigation scenario (RCP2.6), intermediate scenarios (RCP4.5 and RCP6.0) and a very high GHG emissions scenario (RCP8.5). Scenarios include the range of 2100 radiative forcing levels of 8.5, 6, 4.5 and 2.6 W/m² for 1870 (Table 3.3). (van Vuuren et al., 2011; Pachauri and Meyer, 2014).

Table 3.3. Overview of RCP Scenarios (van Vuuren et al., 2011)

Scenario	Description	Citation
RCP 8.5	Rising radiative forcing pathway leading to 8.5 W/m ² (~1370 ppm CO ₂ eq) by 2100.	(Riahi et al. 2007)
RCP 6	Stabilization without overshoot pathway to 6 W/m ² (~850 ppm CO ₂ eq) at stabilization after 2100	(Fujino et al. 2006; Hijioka et al. 2008)
RCP 4.5	Stabilization without overshoot pathway to 4.5 W/m ² (~650 ppm CO ₂ eq) at stabilization after 2100	(Clarke et al. 2007; Smith and Wigley 2006; Wise et al. 2009)
RCP 2.6	Peak in radiative forcing at ~3 W/m ² (~490 ppm CO ₂ eq) before 2100 and then decline.	(Van Vuuren et al., 2007a; van Vuuren et al. 2006)

The overview of the RCP Scenarios and related publications are given in Table 3.3. RCP 8.5 scenario is the worst-case scenario since the RCP 8.5 scenario represents a high greenhouse gas concentration at the end of the century, which is also called a high-emission business as usual. (Riahi et al., 2011). RCP 4.5 is a stabilization scenario and therefore supposed to achieve the goal of limiting greenhouse gas emissions and the anthropogenic components of radiative forcing (Thomson et al., 2011).

3.1.4. CORDEX & Domain Used in This Study

Coordinated Regional Climate Downscaling Experiment (CORDEX) has been founded by World Climate Research Program (WCRP) and developed to provide a better framework for Regional Climate Downscaling (RCD)-related research and modelling activities within the regional climate modelling and downscaling communities. CORDEX has divided the world into 14 regions. These regions are respectively South America, Central America, North America, Europe (EURO), Africa, South Asia, East Asia, Central Asia, Australasia, Antarctica, Arctic, Mediterranean (MED), Middle East and North Africa (MENA), South East Asia (SEA) (CORDEX, 2015; NASA Jet Propulsion Laboratory, 2019). The domain of this study is Region 13: Middle East North Africa (MENA) (Figure-3.2) but it is very large and includes desert parts of Africa, so it is divided into the subdomain, which contains the Mediterranean Basin region. It has the latitudes from 30°N to 50°N and longitudes from -12°E to 53°E.

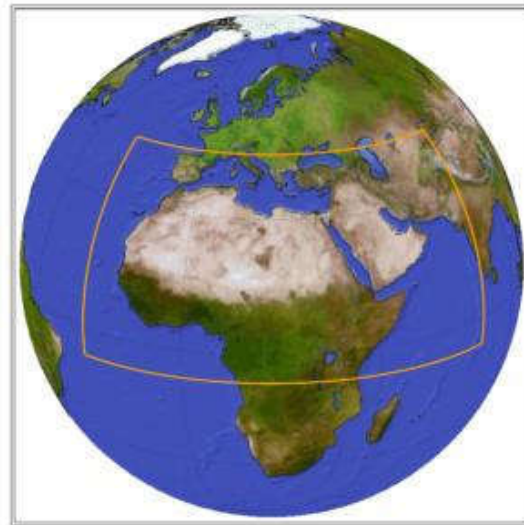


Figure 3.2. CORDEX Region 13: MENA (CORDEX, 2015)

3.1.5. Subdomains Used in This Study

The domain of this study, MENA, is large so it is divided into 5 subdomains to measure average FWI values of chosen areas. The latitudes and longitudes of the subdomains are listed below. These subdomains are shown on ERA-Interim re-forecast map of MENA in Figure 3.3 which is drawn using by R-studio

- Region-1 contains Aegean and Western Mediterranean of Turkey. It has the latitudes from 36°N to 40°N and longitudes from 26°E to 32°E.
- Region-2 contains The Black Sea Region of Turkey. It has the latitudes from 40.12°N to 42.18°N and longitudes from 31°E to 42.50°E.
- Region-3 contains Greece. It has the latitudes from 36.31°N to 41.65°N and longitudes from 19.55°E to 26.30°E.
- Region-4 contains the area of Northern Italy, Southern Switzerland, and Southern France. It has the latitudes from 42.75°N to 46.96°N and longitudes from -1.90°W to 13.85°E.
- Region-5 contains The Iberian Peninsula. It has the latitudes from 36°N to 44°N and longitudes from -9.9°W to 3.5°E.

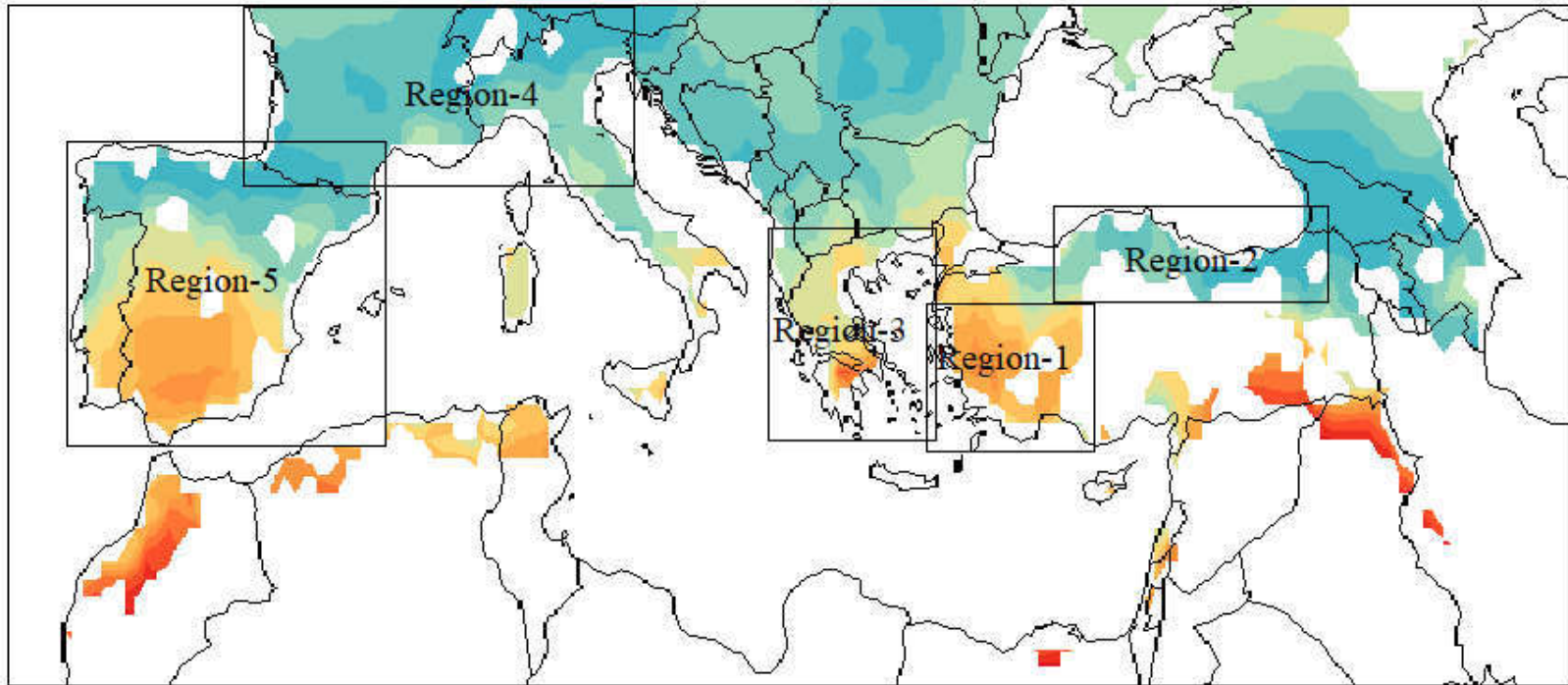


Figure 3.3. Subdomains used in the study

3.1.6. R Studio – FireDanger Package

In this study, the R-packages of fireDanger, transformer, visualizer, easyVerification developed by Santander Meteorology group were used for the calculation of FWI. Moreover, ncd4, lattice, RcolorBrewer and chron libraries in R Studio are other components used to calculate wildfire danger assessment.

3.2. Greenhouse Experiment

3.2.1. Soil Sampling

In the greenhouse set up, two different types of soils were used. The first type of experimental soil used in this study was taken from a mine tailing area in Kütahya(Figure 3.4). In the study, mine soil was utilized to indicate the mining area could be afforested by using arbuscular mycorrhizal fungi (AMF). Thus, the afforested area could protect soil moisture under drought weather conditions in the future. This could decrease potential forest fire risk in there.



Figure 3.4. Kütahya is shown as red in the Turkey map

Other soil was collected from Muğla forest area (Figure 3.5). Muğla soil was used to indicate soil moisture could be raised in forest areas by AMF inoculation.

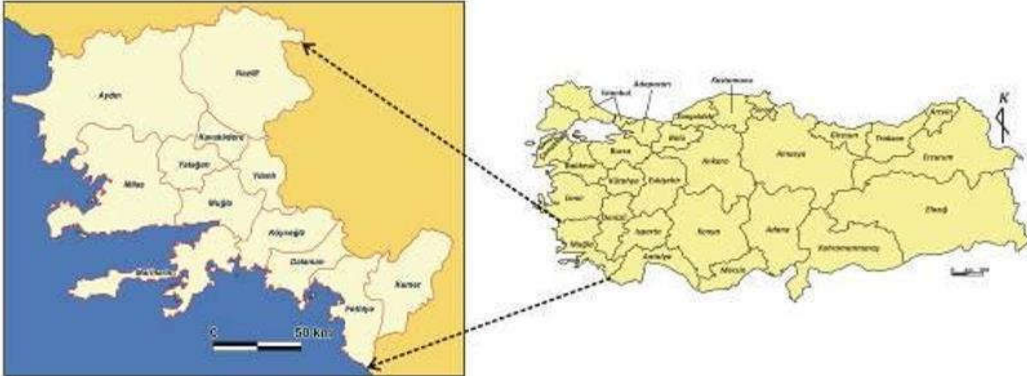


Figure 3.5. Responsibility map of the Regional Directorate of Forestry of Muğla and its affiliated directorates (Türkeş and Altan, 2012)

3.2.2. Metal Analysis of the Soil

The experimental soil samples from Muğla and Kütahya were taken as 0.25 gram as triplicate and digested. The concentrations of Cr, Mn, Fe, Ni, Cu, Zn, Al, Cd, Pb, Si, Co, and Mo in the soil were decided via ICP-OES method after the digestion of soil samples.

3.2.3. Greenhouse Set Up

10 liters volume of plastic rectangular pots, *Pinus nigra* (Turkish pine) saplings, *Quercus* (oak) saplings and *Glomus mosseae* (Arbuscular Mycorrhizal Fungi (AMF)) were used in the greenhouse experiment. The experiment lasted more than three months between July 24th – October 19th. Soil moisture, pH, AM (salt content) measured two times a week for 13 weeks.

Before starting the greenhouse experiment, 1 liter of AMF mixture was prepared with distilled water and, 2g (12 million of spores) of AMF (*Glomus mosseae*) and 100mL AMF mixture given to each sapling in the four pots. AMF (-) pot cultures were non-mycorrhizal groups (also called the control group). AMF (+) pot cultures were mycorrhizal groups. Before starting the greenhouse experiment, AMF mixture was prepared with distilled water (12 million of spores of *Glomus mosseae*) and a 100mL AMF mixture was only given to each sapling in AMF (+) pots.

Greenhouse study was done as Figure-3.6 & Figure-3.7:

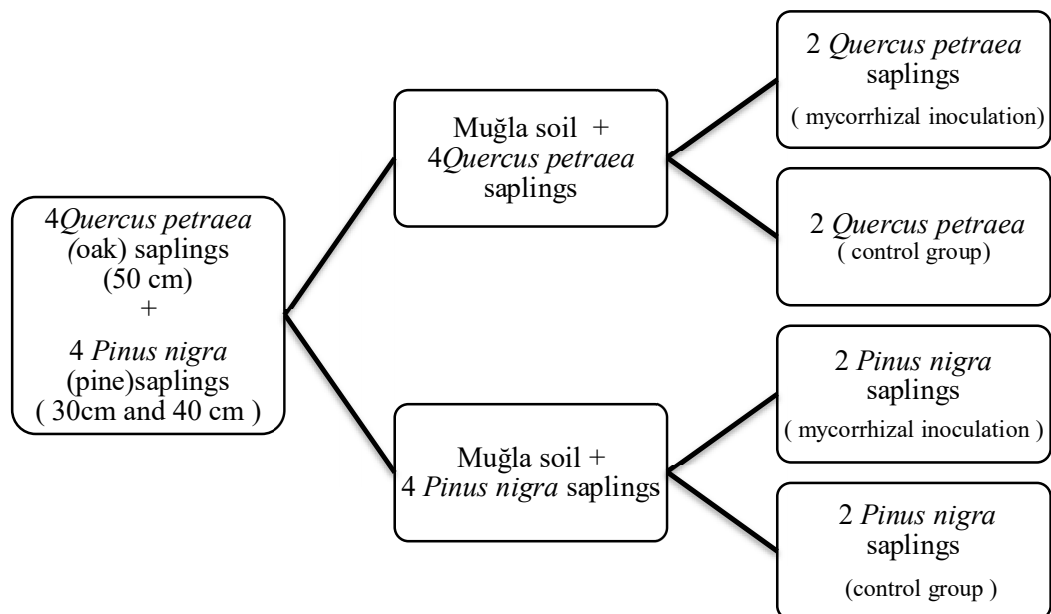


Figure 3.6. Greenhouse Muğla soil set up chart

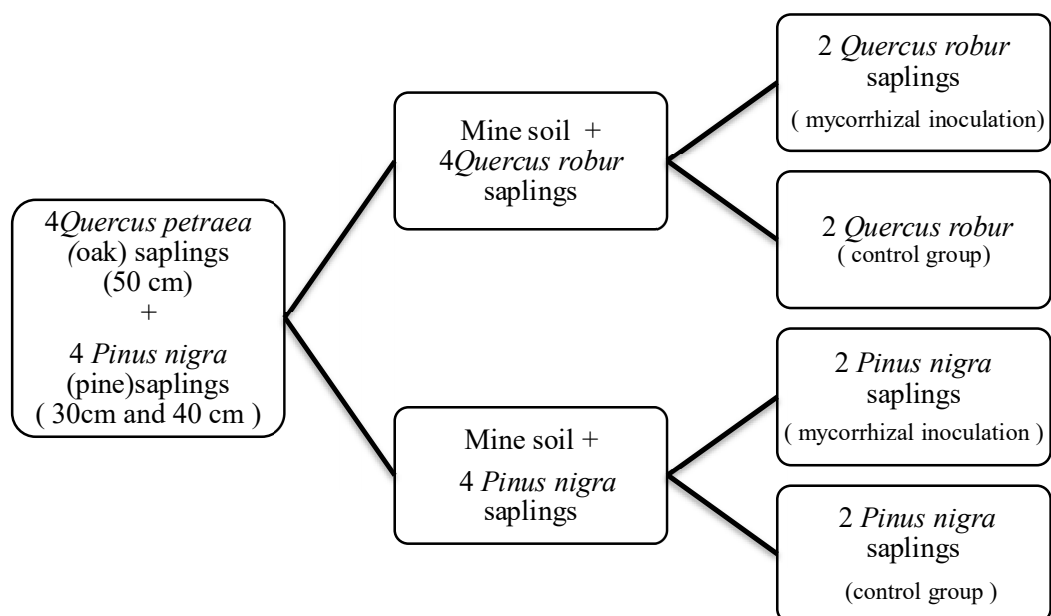


Figure 3.7. Greenhouse mine soil set up chart

100 mL nutrient solution that is called as Modified Strullu-Romand (MSR) medium (Table 3.4) was prepared and added to each pot a month after the plantation and then a 100 ml nutrient solution was added to each pot a week after the first nutrient adding. After starting the greenhouse experiment in July, the nutrient solution was given to each sapling (there were two saplings in each pot) as a mix of 50mL nutrient + 50mL distilled water on the 4th week and 12th week two times during the experiment.

Table 3.4. Composition of MSR medium (Fortin et al., 2002)

Composition	Component	Concentration (mgL ⁻¹)
macro-elements	MgSO ₄ .7H ₂ O	739
	KNO ₃	76
	KCl	65
	KH ₂ PO ₄	4.10
micro-elements	MnSO ₄ .4H ₂ O	4.90
	ZnSO ₄ .7H ₂ O	0.56
	H ₃ BO ₃	3.70
	CuSO ₄ .5H ₂ O	0.44
	Na ₂ MoO ₄ .2H ₂ O	0.0048
	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.068
calciumnitrate	Ca(NO ₃) ₂ .4H ₂ O	359
chelating agent	Na ₂ EDTA	8.7

In many countries of Mediterranean Basin and Turkey, especially western and southern regions, wildfires are at the top of the factors that threaten forests since the summers are very hot and arid, the winters are warm and rainy in the Mediterranean climate zone (Türkeş and Altan, 2012; Erkan, 2006; Altan, 2011; Altan et al., 2011). Muğla is one of the too risky areas of Turkey regarding forest fires due to the arid climate. The high number of forest fires are seen in Muğla during the period between

the months of June and September (Türkeş and Altan, 2012). For this reason, the greenhouse study was based on the microclimate of Muğla. During the experiment, saplings were watered according to the average monthly total rainfall (mm) of Muğla between 1926 – 2017 years (Tarım ve Orman Bakanlığı Meteoroloji Genel Müdürlüğü, İllere ait mevsim normalleri. Ölçüm periyodu (1926 – 2017)).

Rainfall is measured in liters per square meter, which is equivalent to mm (Ankara Üniversitesi, Açık ders malzemeleri, Yağış). In the experiment, the total amount of water used for irrigation was calculated based on a square meter of pots and the average monthly total rainfall of Muğla (Table 3.5). After starting the greenhouse experiment, 1200 mL tap water was used for irrigation between 24 and 31 July. 400 mL tap water for each pot, except mine soil-oak AMF (+) and AMF (-) pots, was added two times a week after soil moisture measurement to prevent drying of the saplings.

Table 3.5. Average monthly total rainfall (mm) of Muğla between 1926 – 2017 years

Muğla	July	August	September
Average monthly total rainfall (mm)	8.4	9.4	19.0

Saplings of mine soil-oak AMF (+) and AMF (-) pots dried just before the experiment began, so new oak saplings were planted in the same mine soil pots at the second week of experiment and 1.7 liters of tap water was added to pots after plantation (Figure 3.8). 0.4 grams of *Glomus mosseae* and 200 mL distilled water were mixed and a 100mL AMF mixture was given to each sapling in mine soil-oak AMF (+) pot to compensate for the loss of AMF.



Figure 3.8. Saplings of mine soil-oak AMF (+) and AMF (-) (control group) (left side) and new oak saplings in same soils (right side)

Additionally, one sapling in the pot of Muğla soil-*Pinus nigra* AMF (-) dried, and then this sapling was replaced with new *Pinus nigra* sapling at 2nd week of experiment and 950 mL tap water was added after replacement (Figure 3.9).



Figure 3.9. Dried sapling in Muğla soil- *Pinus nigra* AMF (-) (control group) pot (left side) and new sapling in same pot (right side)

In August 1400 mL tap water was used for watering. 200 mL of water was given to each pot twice a week after soil moisture, pH and AM measurements were done. 1900 mL of tap water was used in September. 800 mL, 400 mL, 400 mL and 300 mL of water were respectively given to the pots once a week during this month. In October 600 mL of tap water was added to the pots and in the 12th week of the experiment, the nutrient solution (200 mL) was given to the pots.

The temperature of the greenhouse environment was controlled by 3 halogen lights. The lights were started to use to increase greenhouse temperature in September and October. In the greenhouse, the average maximum temperature was 30.43 °C and the average minimum temperature was 22.18 °C. The highest and lowest temperatures of the greenhouse were recorded via the greenhouse thermometer (Figure 3.10).

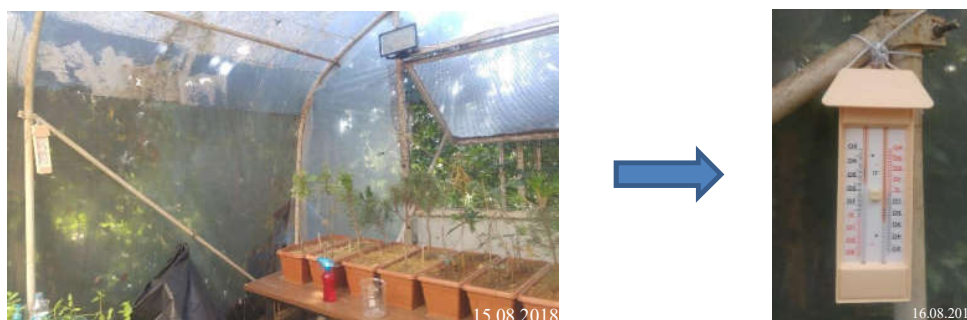


Figure 3.10. The greenhouse thermometer used in the greenhouse experiment

3.2.4. Soil Moisture, pH & Salt Activity Measurements

During the greenhouse experiment, soil moisture, temperature, pH, salt content with activity meter (AM) of eight pots were measured two times a week by using the COMBI 5000, which is a multi-functional device.

3.2.5. Sampling

Root and soil samples (below roots) were taken from eight pots at the end of the experiment. Root samples of each pot were taken for AMF symbiosis observation by microscope and stored in 75% ethanol solution at 4°C until root staining. Soil samples were taken from each pot for Glomalin (GRSP) determination and were packaged separately at -20°C.

3.2.6. Soil Electrical Conductivity Measurements

4 gram of soil sample was taken from each soil samples at the end of the experiment. 40 mL of distilled water (1w soil:10mL distilled water) was added in falcon tubes. The tubes were mixed well and waited in room temperature for one day. Then all samples were centrifuged at 4000 rpm for 20 minutes. The electroconductivity (EC) of soil was measured from the liquid phases by using COMBI 5000 with the EC probe.

3.2.7. Determination of Mycorrhization

The root samples preserved in 75% ethanol solution at 4°C were used to decide the level of mycorrhization. Firstly, roots were packed in tulle and then kept in 10% KOH solution (w/v) for 30 minutes in the autoclave at 121°C. After root cleaning, roots were contacted with 1% HCl solution

for 3 minutes and rinsed with distilled water. The packed roots in the falcon tubes were transferred to 0.05% (w/v) trypan blue solution (1:1:1 lactic acid, glycerol, and water) for one week (Phillips and Hayman, 1970; Brundrett et al., 1996).

The packed roots were opened and aligned on glass slides after the staining process. Cover slides were closed on the slides and fixed by transparent nail polish. The slides were observed under the microscope.

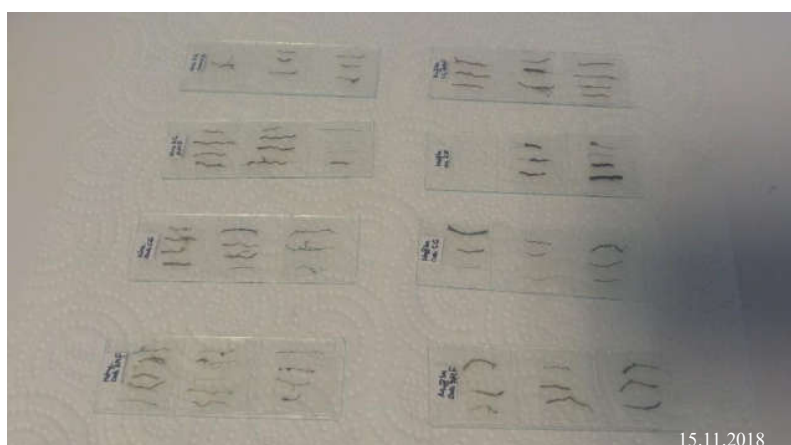


Figure 3.11. Aligned roots on slides for determination of mycorrhization

3.2.8. Measurement of Glomalin Related Soil Protein (GRSP)

1 gram of soil sample from each pot was taken from soil preserved at $-20\text{ }^{\circ}\text{C}$, added 8mL of 50mM Citrate Buffer (pH=8) to remaining soils in 50mL falcon tubes and autoclaved at $121\text{ }^{\circ}\text{C}$ for 60 minutes. Subsequently, all samples were centrifuged at 7000 rpm for 30 minutes. After centrifugation of samples, the supernatants were carefully taken and transferred the liquid to 15mL sterile falcon tubes and kept at $+4\text{ }^{\circ}\text{C}$ until analyzing with a spectrophotometer (Wright and Upadhyaya, 1998; Rillig, 2004). Before the analysis of GRSP, the spectrophotometer was calibrated with the standard solution. Thus, the concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1 mg/mL protein standard solution (P0834-10x1mL; Sigma; 2mg/mL; Lot SLBS3852) were prepared with distilled water (1mL solution) for calibration.

100 μL of each soil extracts (the supernatants) and 3 mL Bradford Reagent (B6916 SIGMA) were mixed well in hand and waited for 40 minutes. Afterward, all samples were analyzed at 595 nm by UV-160A Spectrophotometer, Shimadzu.

3.2.9. Elemental Analysis of Soil

10 gr soil samples collected from eight pots and original Muğla soil were dried in the oven at 103°C and then were put into desiccator for dry cooling. All dried samples were ground with the blender to have homogeneous fine powder form samples.

The Costech Elemental Combustion System 4010 is an automated combustion analyzer used to determine total carbon (C) and nitrogen (N) in the soil samples. In the elemental analysis, the tin sample cups were used. Firstly, the tare of the tin sample cup was determined and then 1 mg sample was put into the tin sample cup to weight with the analytical balance. The tin cup was folded into a sphere or cube shape using two pairs of forceps. The folded cup was placed onto the weighing pan and was recorded the weight (in mg) on the provided datasheet. Then the sample was put into the Elemental Combustion System to decide the concentration of carbon and nitrogen.

4. RESULTS AND DISCUSSION

4.1. The Canadian Fire Weather Index (FWI) Calculation

Wildfires start to be a significant problem in the Mediterranean region since extreme temperatures and drought weather conditions increase with climate change (Gillet et al., 2004; Pausas, 2004; Flannigan et al., 2006). Therefore, the reliable forecasts of extreme fire seasons started to be crucial for making fire risk management. The Canadian Fire Weather Index (FWI) is one of the most widely used of such indices (Bedia et al., 2017). Besides, FWI is currently used as the official index for fire danger predictions issued by the European Forest Fire Information System (Bedia et al., 2017; EFFIS, 2019 <https://effis.jrc.ec.europa.eu/about-effis/technical-background/fire-danger-forecast/>). In the study, FWI was also preferred to predict fire risk in the MENA region.

The projected changes for the period 2070 - 2099 (long term) in common climatic parameters of precipitation (mm), temperature (°C), relative humidity (%) and wind speed (km/h) concerning the control period 1971 - 2000 were utilized to forecast fire risk by FWI. In the study, the R-package of fireDanger developed by the Santander Meteorology group was applied for the calculation of FWI (Bedia et al, 2017; Bedia et al., 2018).

The outputs of MPI-ESM-MR and HadGEM2-ES were dynamically downscaled to 50 km for the CORDEX-MENA domain with utilize of the RegCM4. The projections were realized according to the RCP4.5 and the RCP8.5 concentration scenarios of the Intergovernmental Panel on Climate Change (IPCC) (Ozturk et al., 2018). Furthermore, observational data from ECMWF ERA-Interim covering the period 1980-2012 were used to evaluate forest fire risk by FWI.

In this study, the 33-year re-forecast of the model (historical hindcast) (1980-2012, the 30-year re-forecast of models (1971-2000) and future forecast of the model (2070-2099) included the period May - October (fire season, MJJASO) for predictions. The target period was decided by making a future forecast with FWI for each month and then were determined the beginning and ending month of the fire season. Moreover, the model mask is a static field with a fraction of land in every grid box. The values are between 0 (grid box is fully covered with grass, desert, tundra, glacier, bog or marsh, sea or ocean, urban, inland water) and 1 (grid box is fully covered forest, shrub areas). A grid box is a forest and shrub area if more than 50% of it is forest and shrub areas. This mask was used to calculate

only forest and shrub areas when computing the FWI. In the maps, colored areas demonstrated forest and shrub areas and others were indicated white color.

The validation of the re-forecast provides an observation of the quality of the estimations depending on their past performance. It helps in the decision-making period at a later operational stage (Goddard et al., 2010, Doblas-Reyes et al., 2013; Bedia et al 2017). Furthermore, ERA-interim is generally used to test the performances of models. The first FWI output was forced by the ERA-interim dataset (Figure 4.1) and then FWI historical hindcasts were calculated by MPI-ESM-MR and HadGEM2-ES models as shown in Figure 4.2 and Figure 4.3.

The results indicate that FWI historical hindcast output by MPI-ESM-MR model matched up with the output of FWI re-forecast by observational data (Figure 4.1, Figure 4.2). On the other hand, FWI historical hindcast by HadGEM2-ES model data was not more like the output of FWI re-forecast by observational data (Figure 4.1, Figure 4.3). This preliminary assessment suggests which model data could provide a skillful FWI forecast in the chosen region in the study.

The outputs of FWI forecasts for the future period 2070-2099 with respect to the reference period 1971-2000 based on the IPCC's RCP4.5 (Figure 4.4, Figure 4.5) and RCP8.5 (Figure 4.6, Figure 4.7) scenarios were presented. It is apparent that the projections demonstrate a serious increase in forest fire risk throughout the region for the last 30 years of this century.

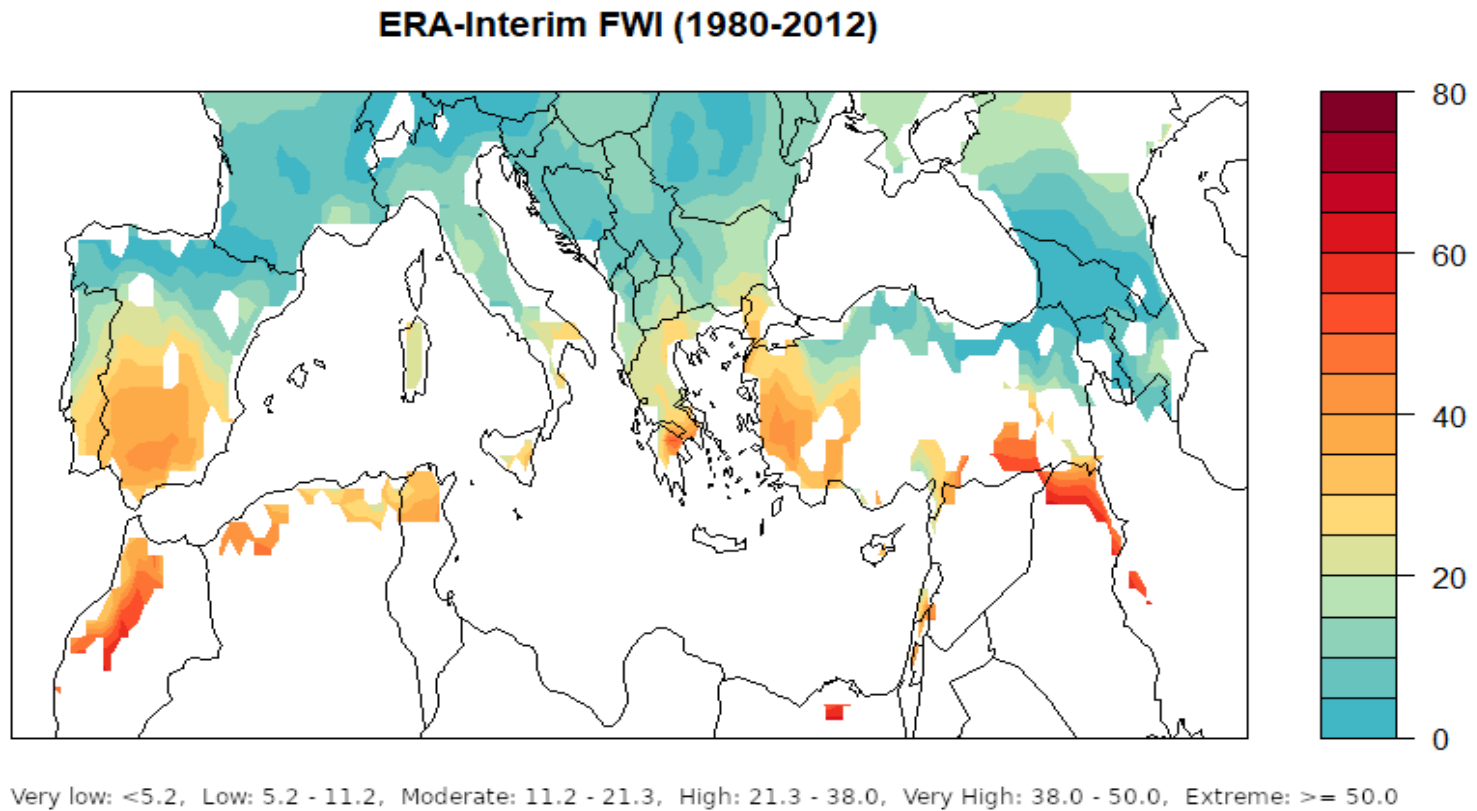
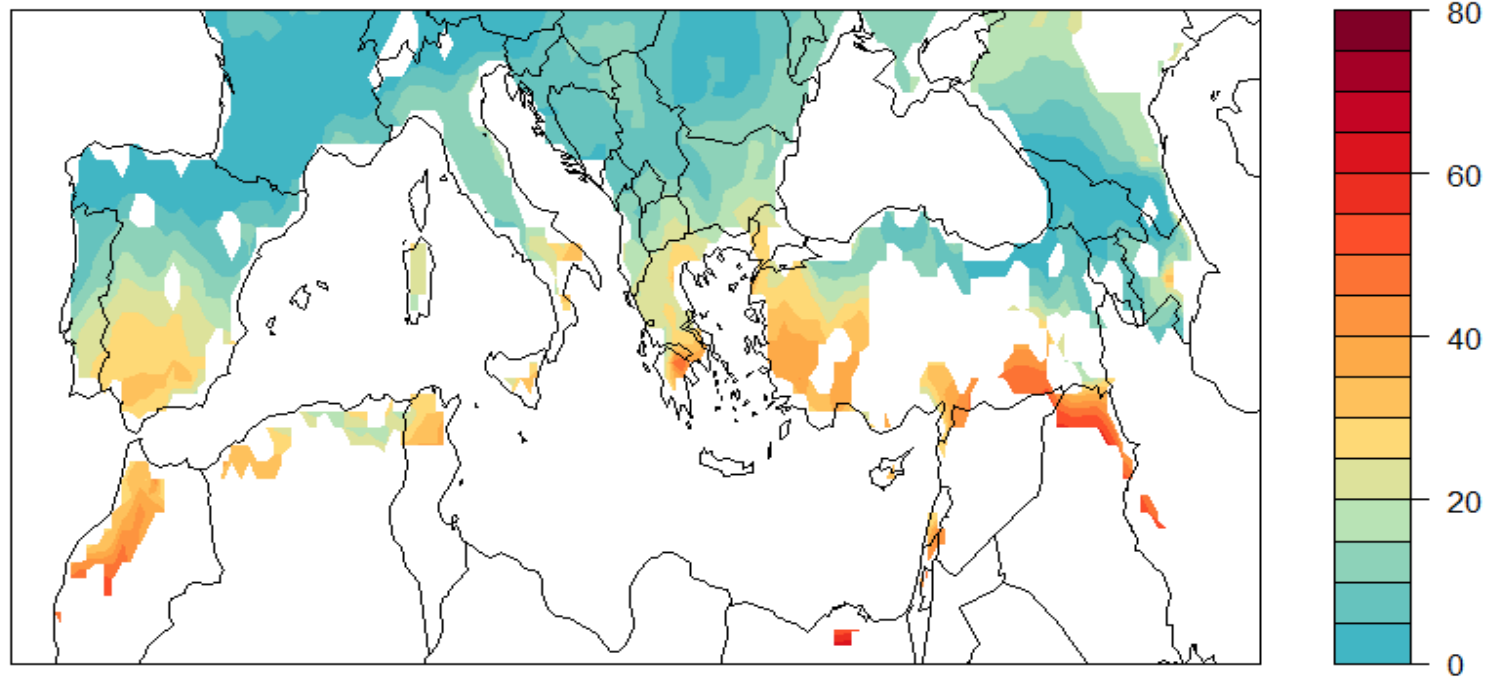


Figure 4.1. Observed FWI climatology, as depicted by the ERA-Interim observational dataset over the period 1980–2012

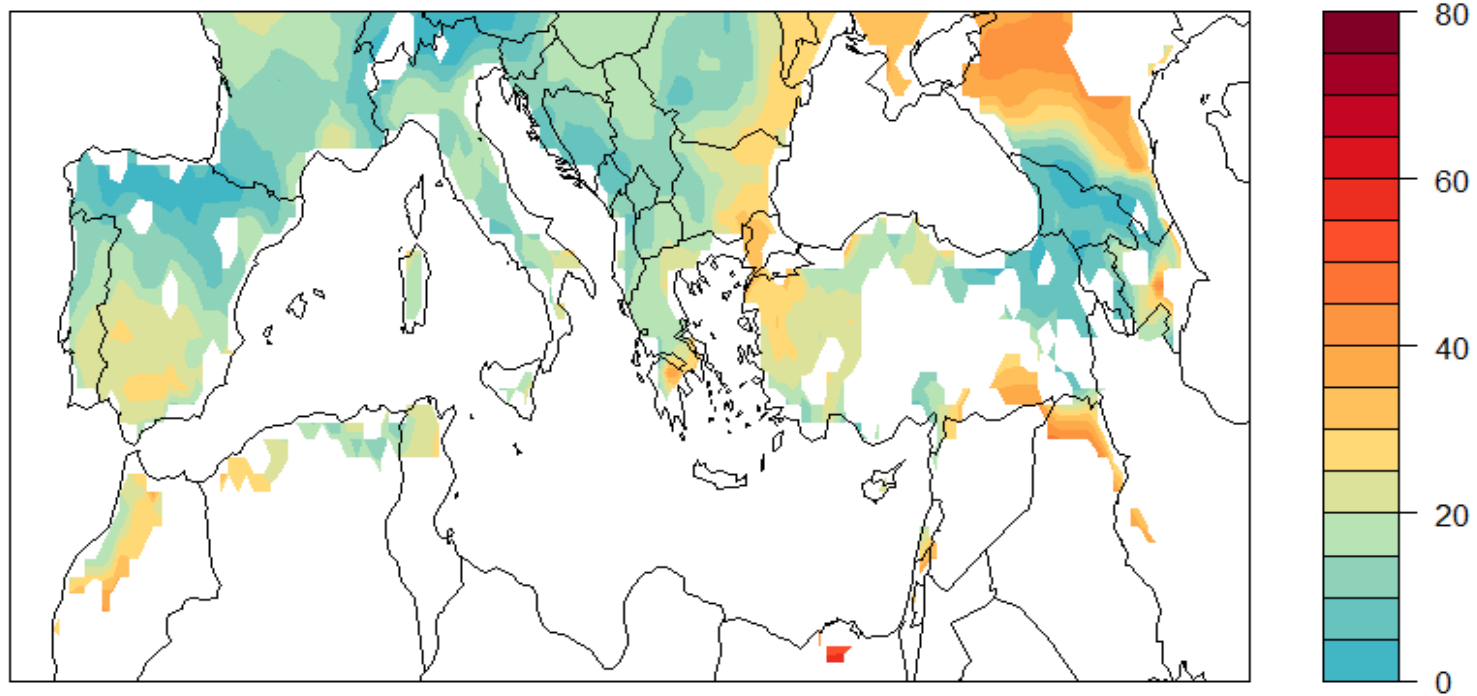
MPI-ESM-MR FWI (1971-2000)



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.2. FWI re-forecast by using MPI-ESM-MR model (the period 1971–2000)

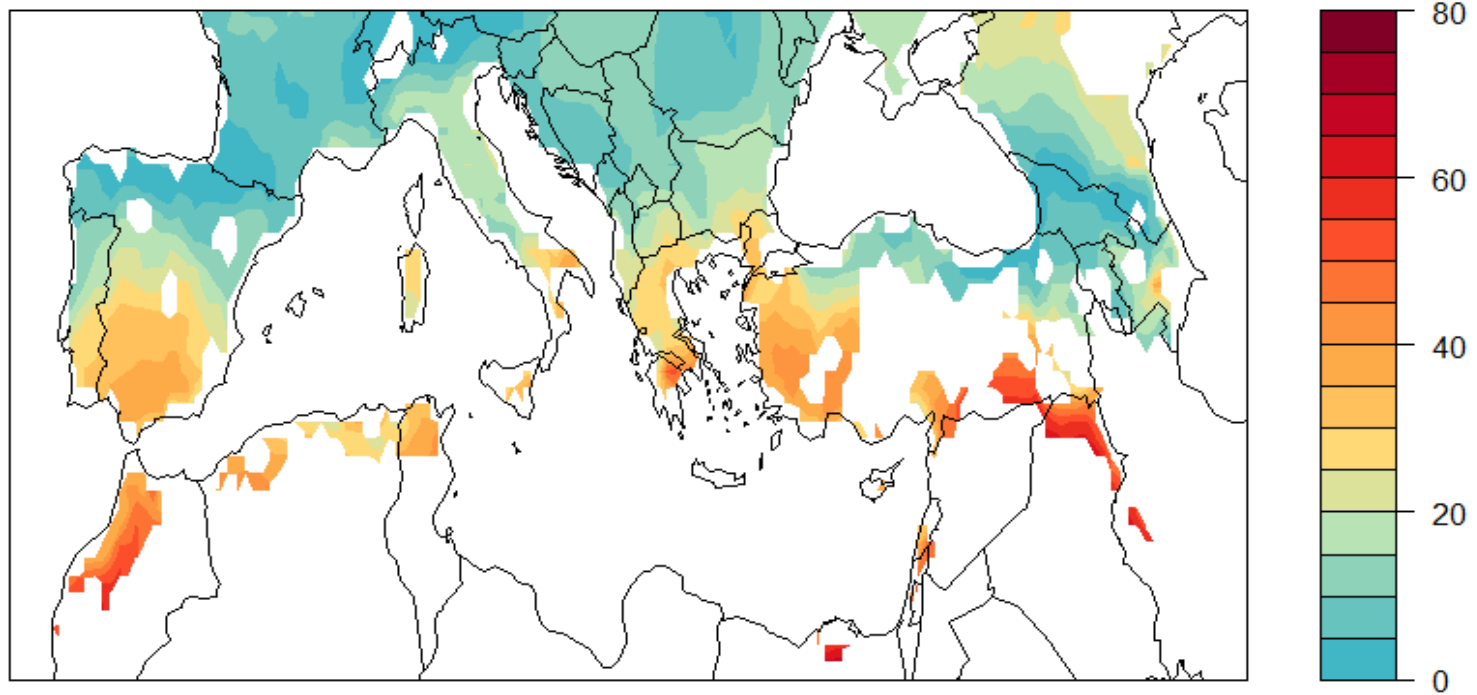
HadGEM2-ES FWI (1971-2000)



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.3. FWI re-forecast by using HadGEM2-ES model (the period 1971–2000)

MPI-ESM-MR RCP4.5 FWI (2070-2099)



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.4. FWI forecast (MJJASO, 2070–2099) by using MPI-ESM-MR model with RCP4.5 emission scenario

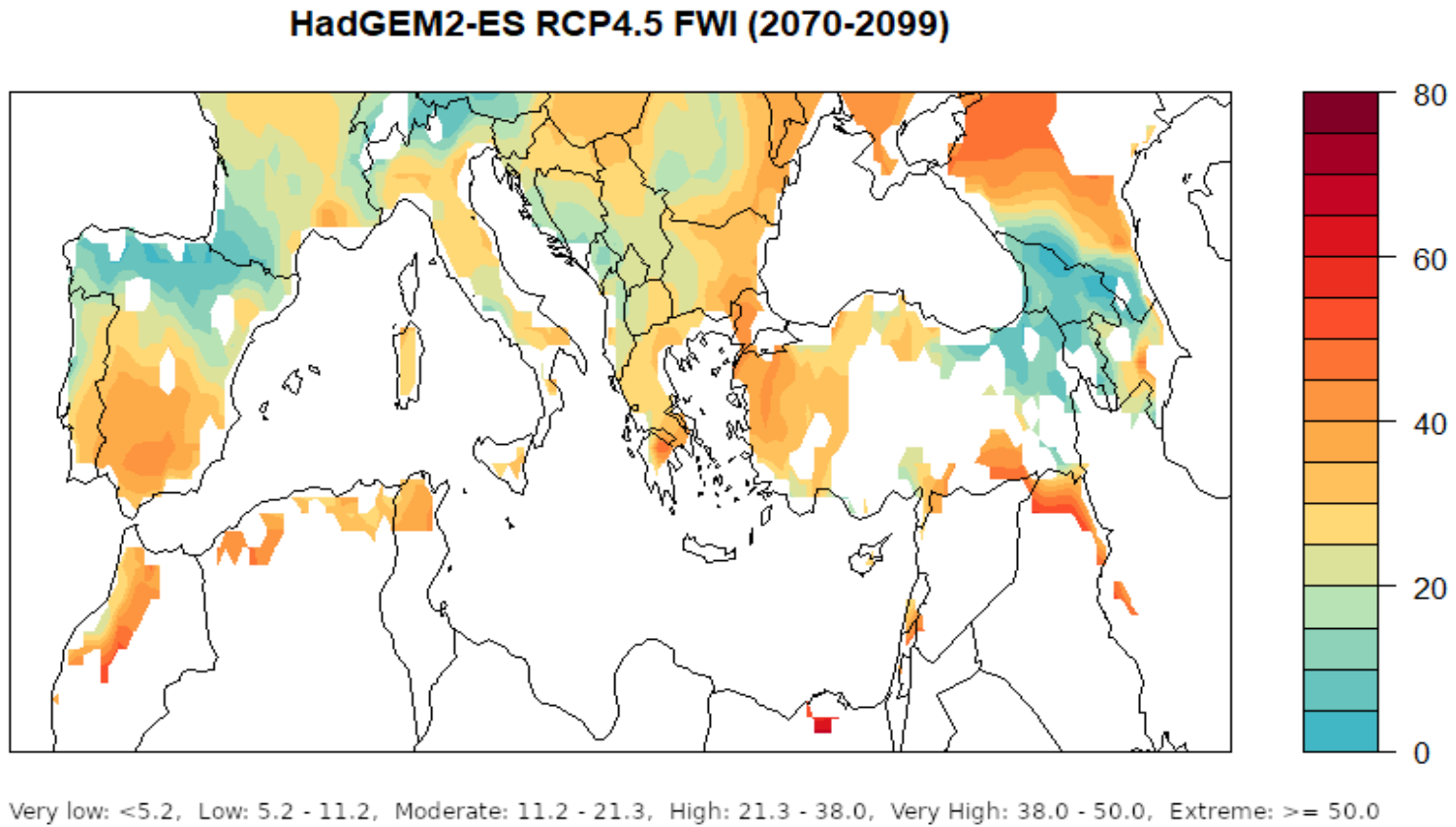


Figure 4.5. FWI forecast (MJJASO, 2070–2099) by using HadGEM2-ES model with RCP4.5 emission scenario

MPI-ESM-MR RCP8.5 FWI (2070-2099)

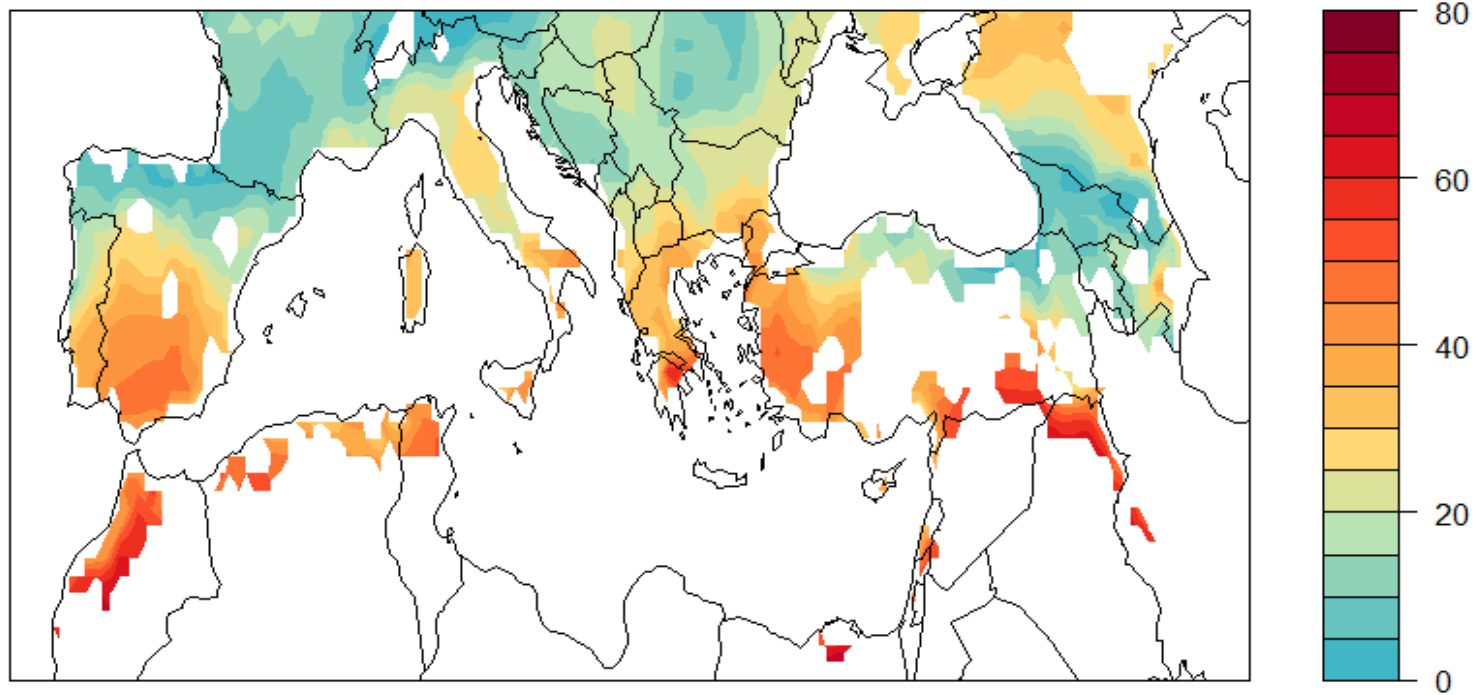
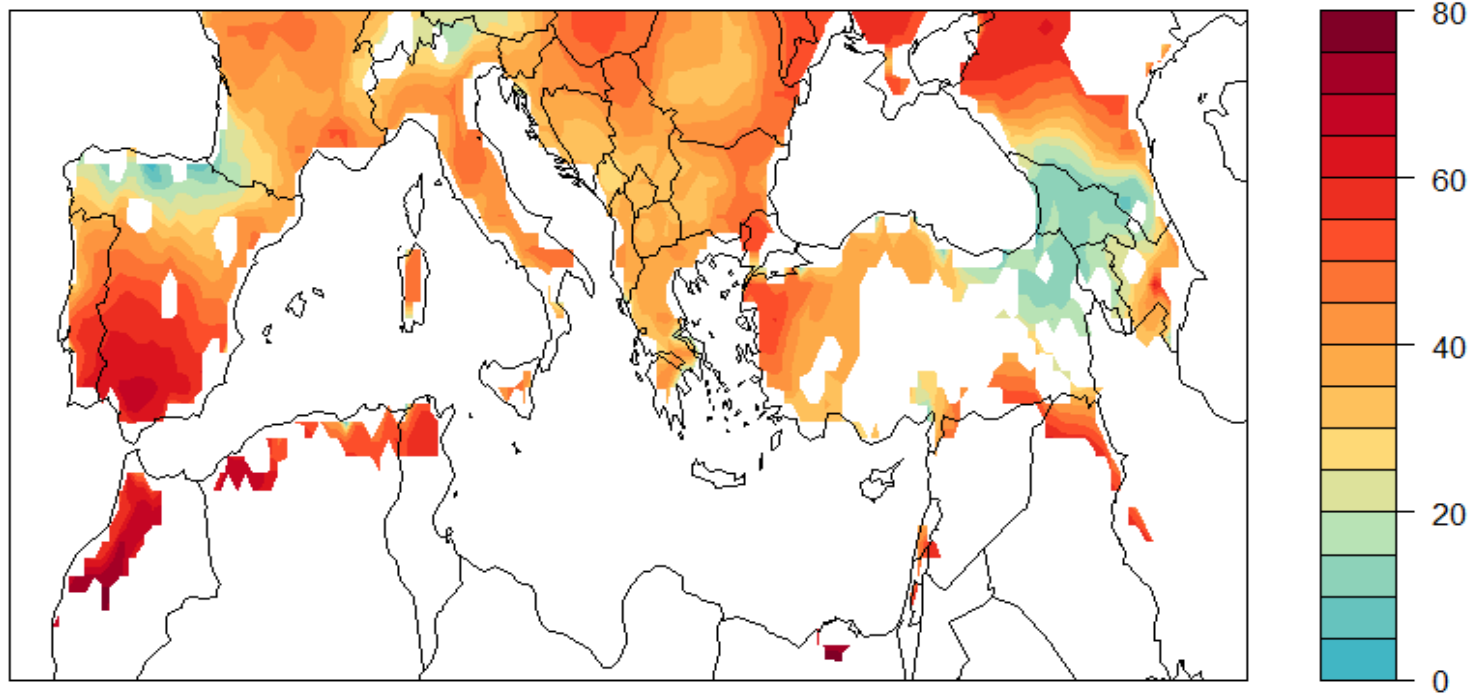


Figure 4.6. FWI forecast (MJJASO, 2070–2099) by using MPI-ESM-MR model with RCP8.5 emission scenario

HadGEM2-ES RCP8.5 FWI (2070-2099)



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

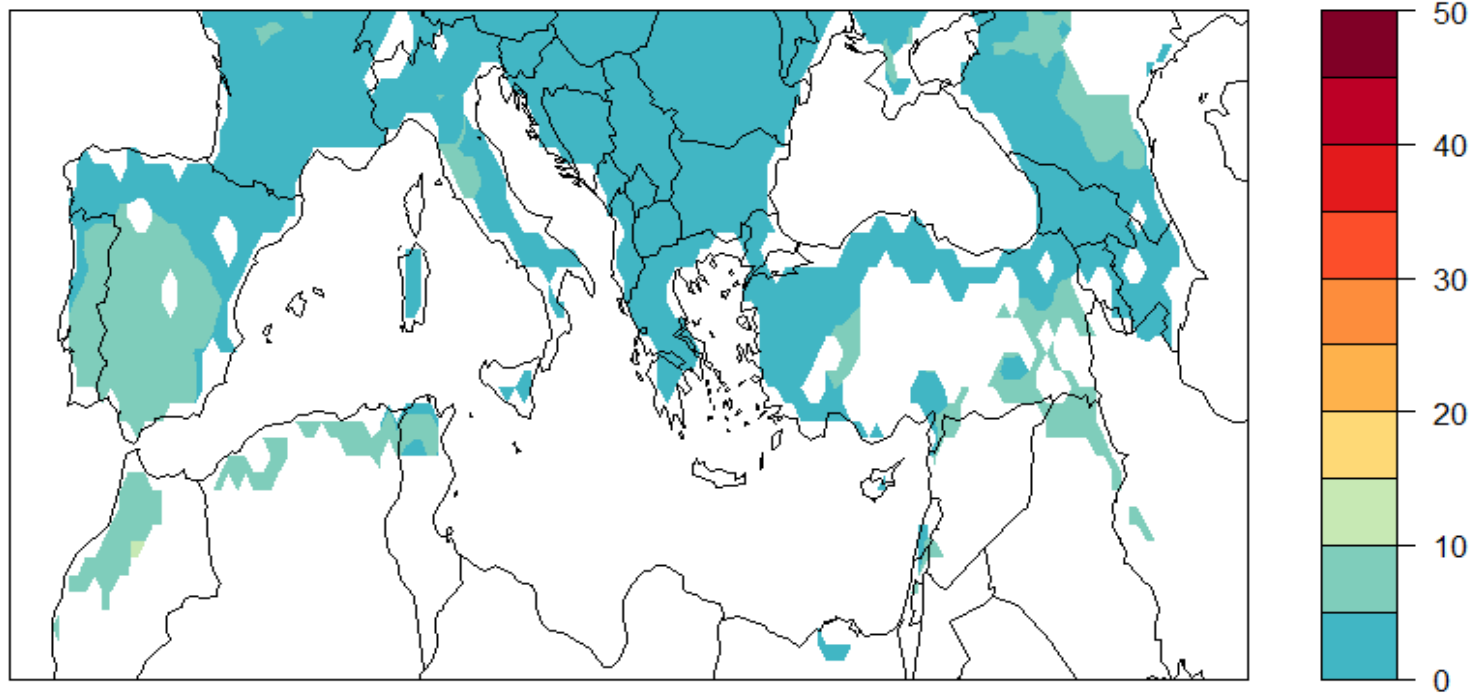
Figure 4.7. FWI forecast (MJJASO, 2070–2099) by using HadGEM2-ES model with RCP8.5 emission scenario

The difference in the output of the FWI forecast (RCP4.5) from the output of FWI historical hindcast indicates how much forest fire risk will increase in the future. According to the difference map, as shown in Figure 4.8, FWI-MPI-ESM-MR projection under the low-concentration scenario RCP4.5 in Greece and the southern and western parts of Turkey, Albania, Macedonia, Southern France, Italy, and Slovenia, where the risk of forest fires demonstrate a low-level increase according to EFFIS FWI classification. Besides, the rise in forest fire risk in the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the Iberian Peninsula (Spain, Portugal) could be identified as low and moderate in the southern part of Spain (Andalusia).

According to the difference map, as shown in Figure 4.9, FWI- HadGEM2-ES projection under the low-concentration scenario RCP4.5 in Greece and the southern and western parts of Turkey, Albania, Macedonia, Southern France, Italy, and Slovenia, where the risk of forest fires demonstrate a moderate-level increase according to EFFIS FWI classification. Besides, the rise in forest fire risk in the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the Iberian Peninsula (Spain, Portugal) could be identified as moderate and high in the southern part of Spain (Andalusia).

All in all, the outputs of FWI forecasts under the low-concentration scenario RCP4.5 which were calculated by MPI-ESM-MR and HadGEM2-ES models demonstrate forest fire risk will increase in the future. The rise in the fire risk was observed more in the FWI output of the the HadGEM2-ES model than in the FWI output of MPI-ESM-MR. Moreover, the forest cover in these regions is also relatively rich with the Mediterranean climate (Tatlı and Türkeş, 2014). Thus, the FWI analysis presents basic information for estimating the burned area and the number of fires under future climate scenarios.

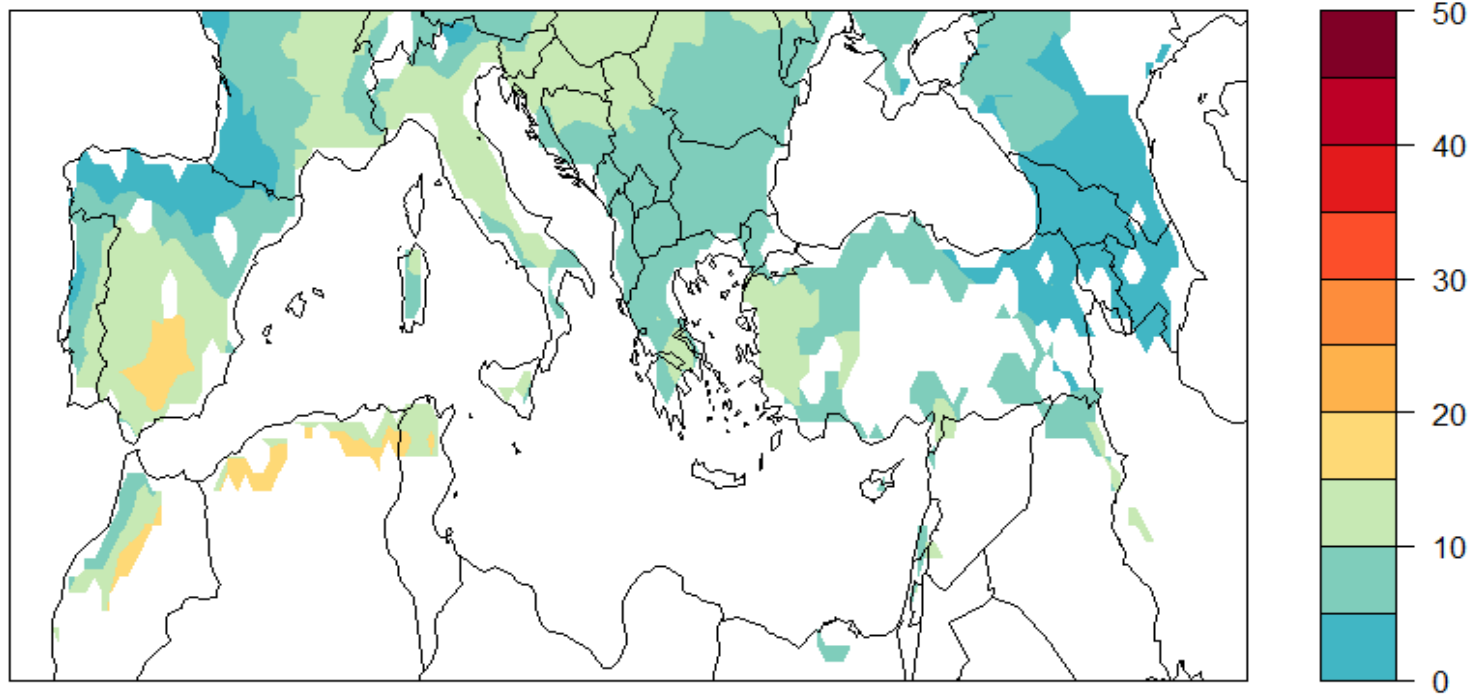
MPI-ESM-MR FWI Diff 2070-2099(RCP4.5) - 1971-2000



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.8. The difference of the output of FWI - MPI-ESM-MR model (2070–2099) with RCP4.5 emission scenario from the output of FWI - MPI-ESM-MR model (1971–2000)

HadGEM2-ES FWI Diff 2070-2099(RCP4.5) - 1971-2000



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

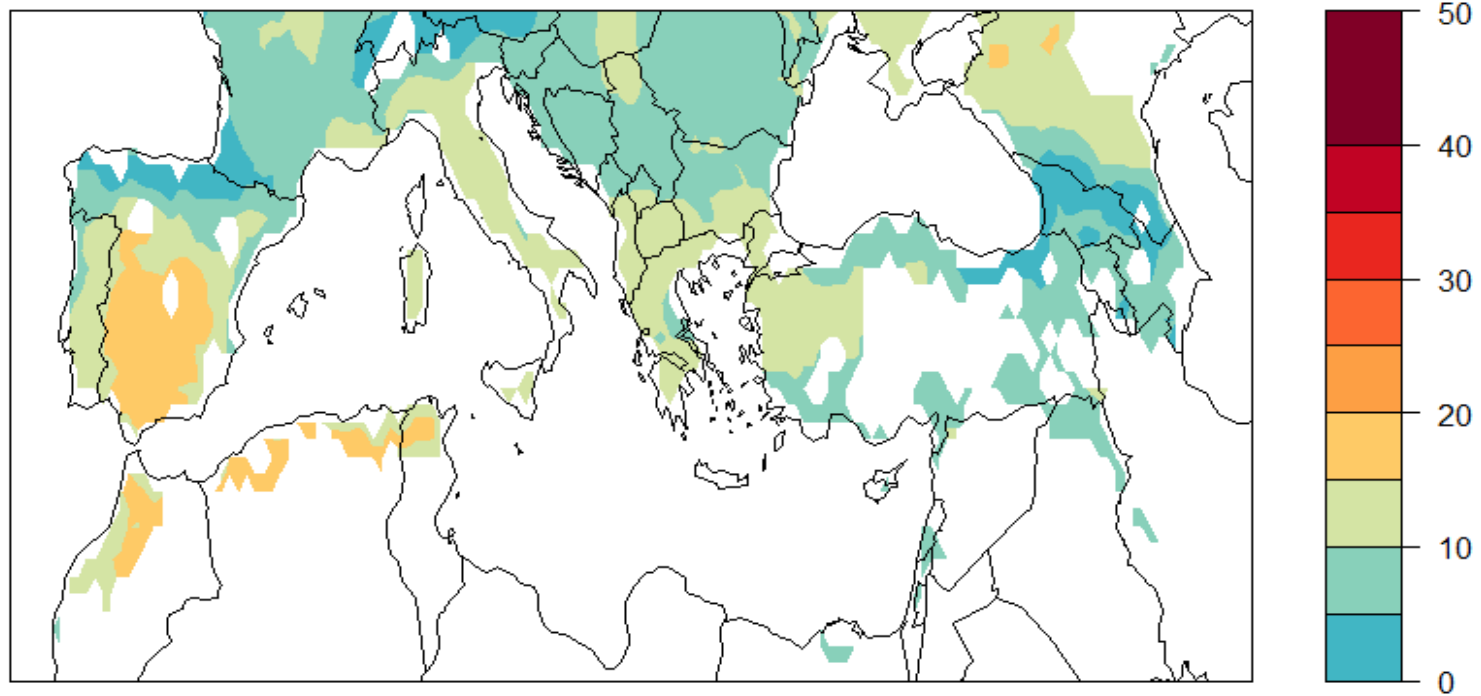
Figure 4.9. The difference of the output of FWI - HadGEM2-ES model (2070–2099) with RCP4.5 emission scenario from the output of FWI - HadGEM2-ES model (1971–2000)

The difference in the output of the FWI forecast (RCP8.5) from the output of FWI historical hindcast indicates how much forest fire risk will increase in the future. According to the difference map, as shown in Figure 4.10, FWI-MPI-ESM-MR projection under the high-concentration scenario RCP8.5 in the southern and western parts of Turkey, Greece, Albania, Macedonia, Southern France, Italy, and Slovenia, where the risk of forest fires frequently demonstrate a moderate-level increase according to EFFIS FWI classification. Besides, the rise in forest fire risk in the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the Iberian Peninsula (Spain, Portugal) could generally be identified as high.

According to the difference map, as shown in Figure 4.11, FWI- HadGEM2-ES projection under the high-concentration scenario RCP8.5 in the southern and western parts of Turkey, Greece, Albania, Macedonia, Southern France, Italy, and Slovenia, where the risk of forest fires demonstrate a high-level increase according to EFFIS FWI classification. Besides, the rise in forest fire risk in the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the Iberian Peninsula (Spain, Portugal) could generally be identified as very high and extreme in the southern part of Spain (Andalusia).

All in all, the outputs of FWI forecasts under the high-concentration scenario RCP8.5 which were calculated by MPI-ESM-MR and HadGEM2-ES models demonstrate forest fire risk will increase in the future. The rise in the fire risk was observed more in the FWI output of the HadGEM2-ES model than in the FWI output of MPI-ESM-MR. Moreover, the forest cover in these regions is also relatively rich with the Mediterranean climate (Tatlı and Türkeş, 2014). Thus, the FWI analysis presents basic information for estimating the burned area and the number of fires under future climate scenarios.

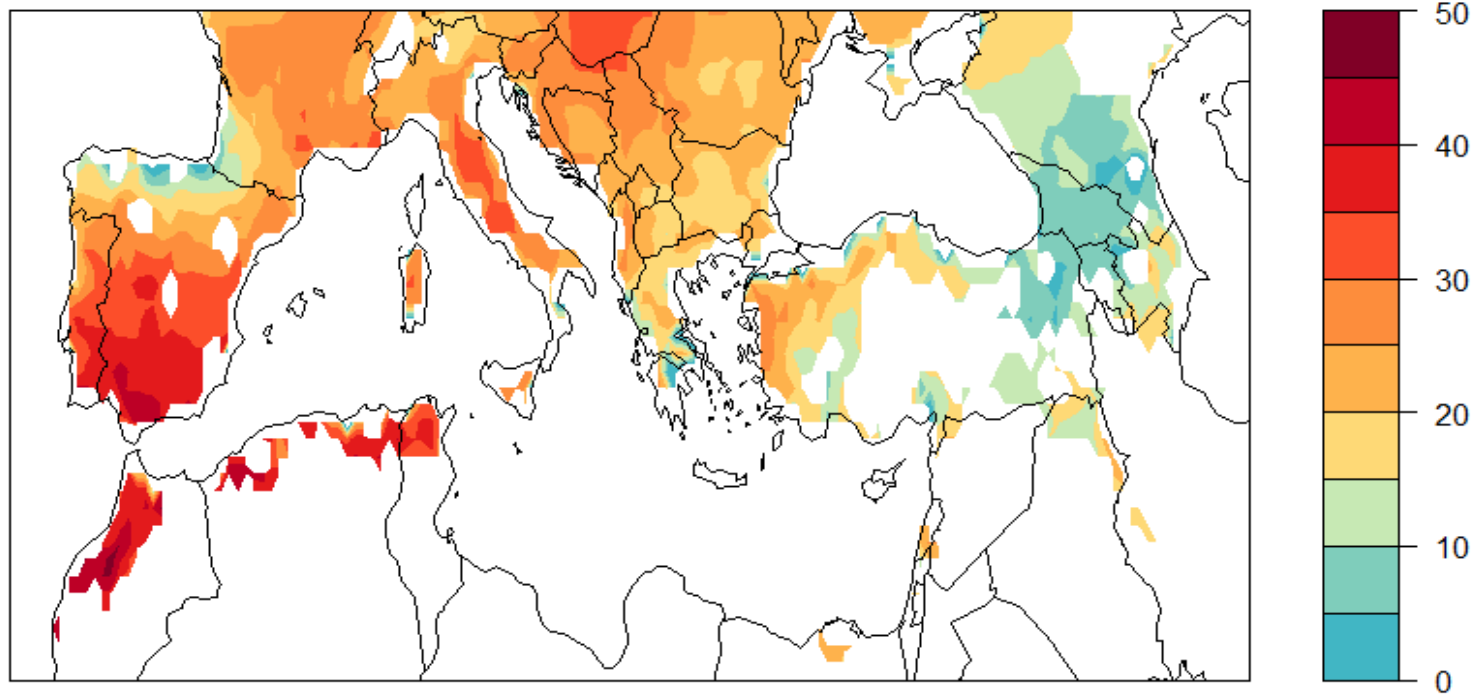
MPI-ESM-MR FWI Diff 2070-2099(RCP8.5) - 1971-2000



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.10. The difference of the output of FWI - MPI-ESM-MR model (2070–2099) with RCP8.5 emission scenario from the output of FWI - MPI-ESM-MR model (1971–2000)

HadGEM2-ES FWI Diff 2070-2099(RCP8.5) - 1971-2000



Very low: <5.2, Low: 5.2 - 11.2, Moderate: 11.2 - 21.3, High: 21.3 - 38.0, Very High: 38.0 - 50.0, Extreme: ≥ 50.0

Figure 4.11. The difference of the output of FWI - HadGEM2-ES model (2070–2099) with RCP8.5 emission scenario from the output of FWI - HadGEM2-ES model (1971–2000)

4.1.1. FWI Results of Region-1

In Figure 4.12, FWI outputs of re-forecasts and forecasts (RCP4.5, RCP8.5) by MPI-ESM-MR and HadGEM2-ES is given for Region-1, which surrounds Aegean and West Mediterranean of Turkey. According to the results of the MPI-ESM-MR model, the average value of FWI historical hindcast is 32.1, which indicates a high-level risk of forest fires for Region-1 concerning EFFIS FWI Classification. Moreover, the average values of FWI forecasts with RCP4.5 and RCP8.5 scenarios by MPI-ESM-MR are respectively 36.0 (high-level fire risk) and 42.5 (very high-level fire risk) in the future. These average values of FWI forecasts demonstrate that in the future, forest fire risk will increase 12% under the low-concentration scenario RCP4.5 and 32% under the high-concentration scenario RCP8.5.

Furthermore, according to the results of the HadGEM2-ES model, the average value of FWI re-forecast is 21.9, which shows a high-level risk of forest fires for Region-1. The average values of FWI outputs with RCP4.5 and RCP8.5 scenarios by HadGEM2-ES are respectively 32.5 (high-level fire risk) and 40.8 (very high-level fire risk). These average values of FWI forecasts demonstrate that the risk of forest fires will raise 48% under the low-concentration scenario RCP4.5 and 86 % under the high-concentration scenario RCP8.5 in the future (Figure 4.12).

Several studies related to wildfires indicate the cities of Muğla, İzmir, Antalya, Aydın are most risky areas in terms of forest fires in Aegean and West Mediterranean of Turkey (Türkeş and Altan, 2012; Kum and Sönmez, 2016; Satır et al., 2016). The results of FWI re-forecasts by MPI-ESM-MR and HadGEM2-ES also provide wildland fires that are a serious factor that threatens forests in the Region-1. Additionally, according to estimates of the FWI outputs of the HadGEM2-ES model, a significant increase in fire hazard is observed, which is more than the prediction results of the MPI-ESM-MR model.

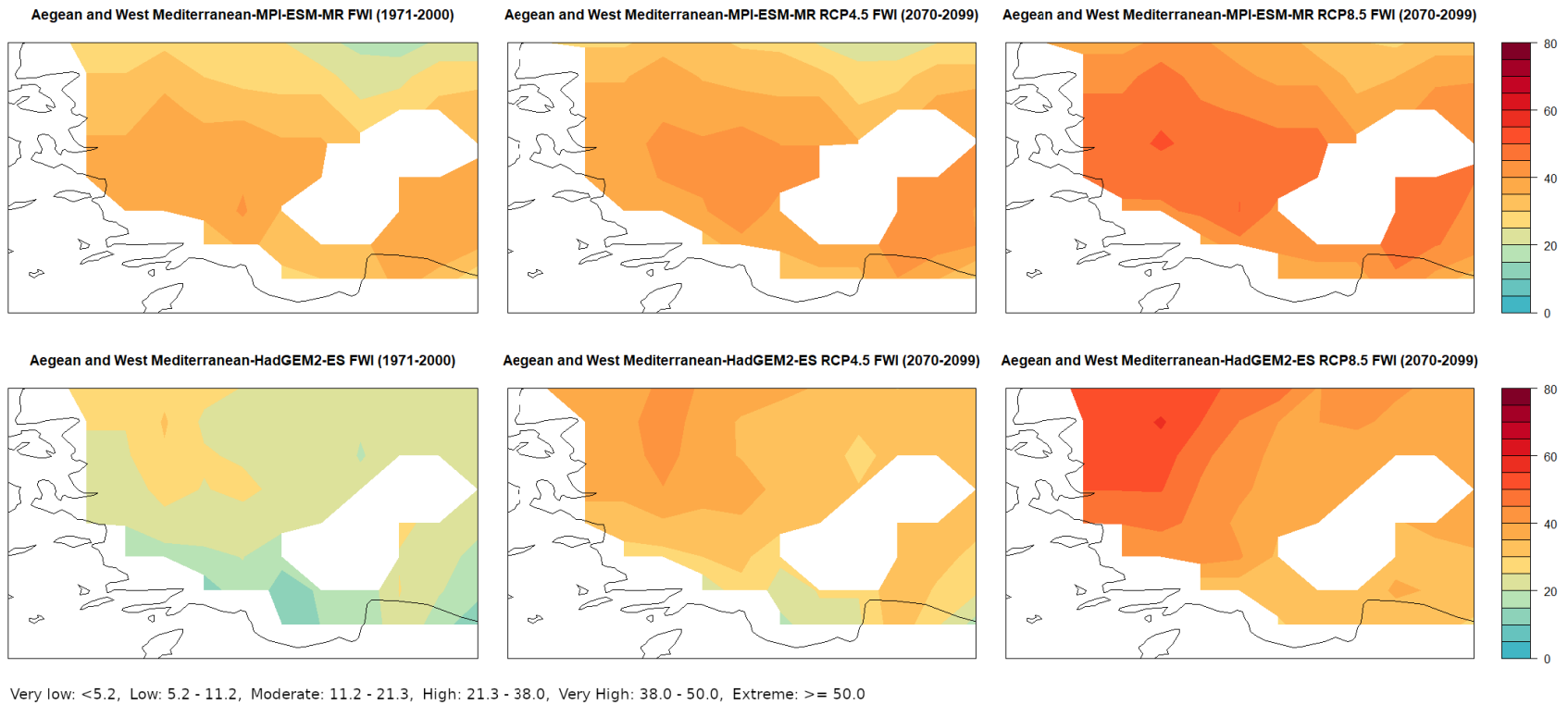


Figure 4.12. The Aegean and the West Mediterranean Regions - the historical hindcast FWI - HadGEM2-ES and MPI-ESM-MR (1971–2000) outputs and the outputs of FWI - MPI-ESM-MR and HadGEM2-ES models (2070–2099) with RCP4.5 and RCP8.5 emission scenarios

4.1.2. FWI Results of Region-2

In Figure 4.13, FWI outputs of re-forecasts and forecasts (RCP4.5, RCP8.5) by MPI-ESM-MR and HadGEM2-ES were given for Region-2, which surround the Black Sea Region of Turkey. According to the results of the MPI-ESM-MR model, the average value of FWI historical hindcast is 8.2, which indicates a low-level risk of forest fires for Region-2 concerning EFFIS FWI Classification. Moreover, the average values of FWI forecasts with RCP4.5 and RCP8.5 scenarios by MPI-ESM-MR are respectively 10.5 (low-level fire risk) and 14.9 (moderate-level fire risk) in the future. These average values of FWI forecasts demonstrate that in the future, forest fire risk will increase 28% under the low-concentration scenario RCP4.5 and 82% under the high-concentration scenario RCP8.5.

Moreover, according to the results of the HadGEM2-ES model, the average value of FWI re-forecast is 14.9, which shows a moderate-level risk of forest fires for Region-2. The average values of FWI outputs with RCP4.5 and RCP8.5 scenarios by HadGEM2-ES are respectively 21.2 (moderate-level fire risk) and 27.4 (high-level fire risk). These average values of FWI forecasts demonstrate that the risk of forest fires will raise 42% under the low-concentration scenario RCP4.5 and 84 % under the high-concentration scenario RCP8.5 in the future.

The results of FWI re-forecasts by MPI-ESM-MR and HadGEM2-ES provide that fire risk in this region is low in terms of the result of MPI-ESM-MR historical hindcast and is moderate according to the outputs of HadGEM2-ES re-forecast. Moreover, the MPI-ESM-MR model result gives a more accurate estimation than the output of the HadGEM2-ES model based on the re-forecast result of observational data (ERA-interim) (Figure 4.1). Additionally, according to estimates of the FWI outputs of the HadGEM2-ES model, a significant increase in fire danger has been seen, which is more than the prediction results of the MPI-ESM-MR model. All in all, forest fires start to be a factor that threatens forests in the Region-2.

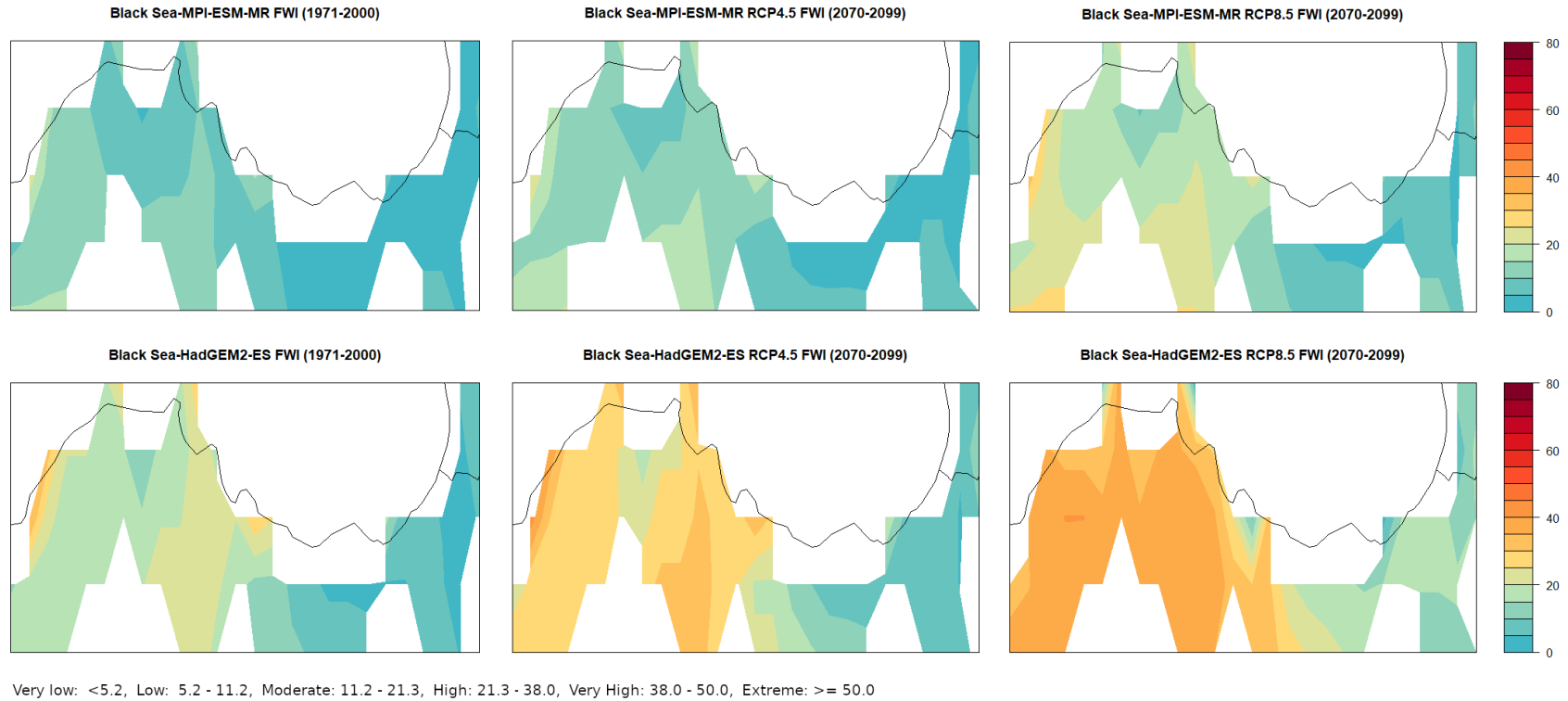


Figure 4.13. The Black Sea Region - the historical hindcast FWI - HadGEM2-ES and MPI-ESM-MR (1971–2000) outputs and the outputs of FWI - MPI-ESM-MR and HadGEM2-ES models (2070–2099) with RCP4.5 and RCP8.5 emission scenarios

4.1.3. FWI Results of Region-3

In Figure 4.14, FWI outputs of re-forecasts and forecasts (RCP4.5, RCP8.5) by MPI-ESM-MR and HadGEM2-ES were given for Region-3, which surround Greece. According to the results of the MPI-ESM-MR model, the average value of FWI historical hindcast is 25.1, which indicates a high-level risk of forest fires for Region-3 concerning EFFIS FWI Classification. Moreover, the average values of FWI forecasts with RCP4.5 and RCP8.5 scenarios by MPI-ESM-MR are respectively 28.4 (low-level fire risk) and 36.1 (moderate-level fire risk) in the future. These average values of FWI forecasts demonstrate that in the future, forest fire risk will increase 13% under the low-concentration scenario RCP4.5 and 44% under the high-concentration scenario RCP8.5.

Additionally, according to the results of the HadGEM2-ES model, the average value of FWI re-forecast is 20.1, which shows a moderate-level risk of forest fires for Region-3. The average values of FWI outputs with RCP4.5 and RCP8.5 scenarios by HadGEM2-ES are respectively 29.2 (high-level fire risk) and 34.9 (high-level fire risk). These average values of FWI forecasts demonstrate that the risk of forest fires will raise 45% under the low-concentration scenario RCP4.5 and 74 % under the high-concentration scenario RCP8.5 in the future.

The outcomes of FWI historical hindcasts by MPI-ESM-MR and HadGEM2-ES provide forest fires that are a significant factor that threatens forests in the Region-3. Moreover, according to predictions of the FWI outputs of the HadGEM2-ES model with the RCP4.5 scenario, a significant increase in fire hazard is observed, which is more than the forecast results of the MPI-ESM-MR model (RCP4.5). On the other hand, the forecast results of the MPI-ESM-MR model under the RCP8.5 scenario indicate a higher increase than the output of the HadGEM2-ES model with the RCP8.5 scenario. Besides, in the future, the number of forest fires will extremely rise in the southern part of Greece (The Peloponnese Peninsula) concerning the FWI forecast of the MPI-ESM-MR model with RCP8.5.

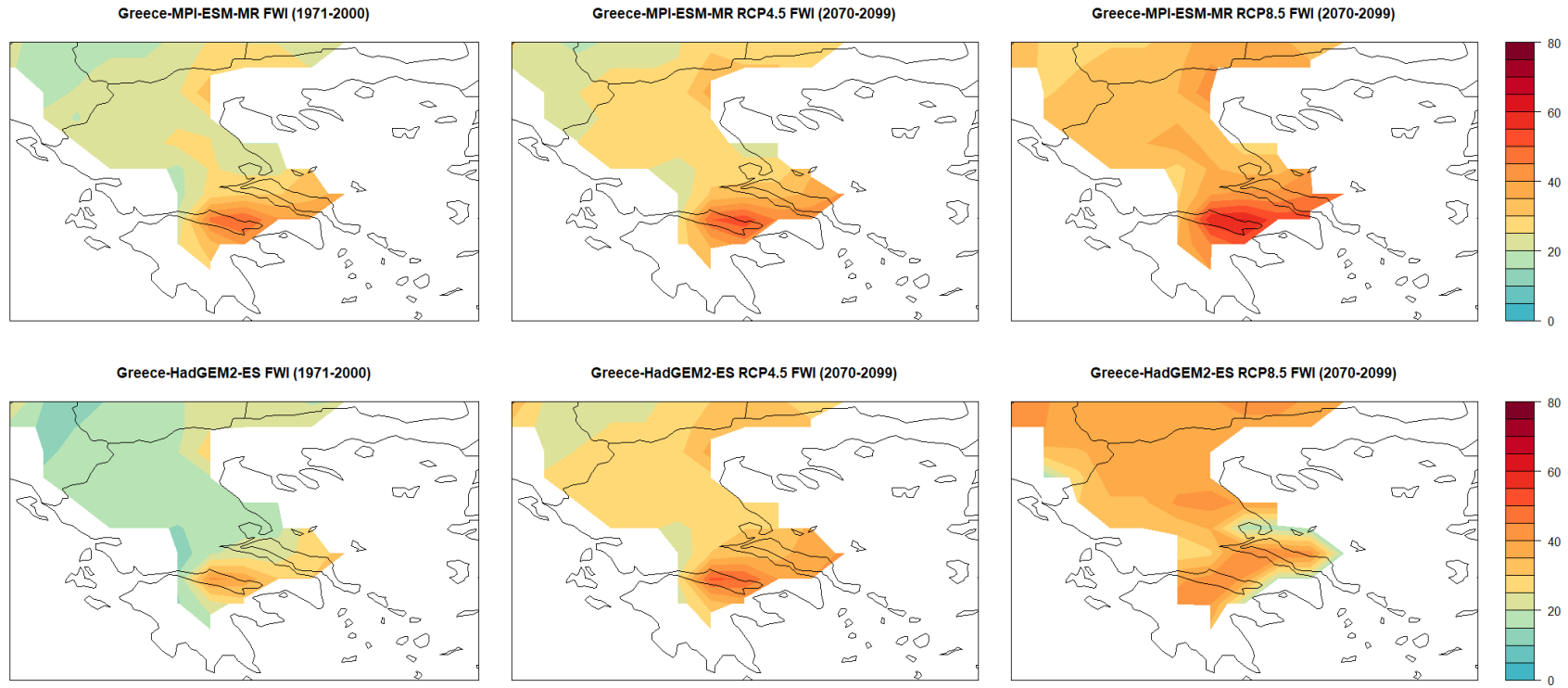


Figure 4.14. Greece - the historical hindcast FWI - HadGEM2-ES and MPI-ESM-MR (1971–2000) outputs and the outputs of FWI - MPI-ESM-MR and HadGEM2-ES models (2070–2099) with RCP4.5 and RCP8.5 emission scenarios

4.1.4. FWI Results of Region-4

In Figure 4.15, FWI outputs of re-forecasts and forecasts (RCP4.5, RCP8.5) by MPI-ESM-MR and HadGEM2-ES were given for Region-4, which surround the region of Southern France, Northern Italy, and Southern Switzerland. According to the results of the MPI-ESM-MR model, the average value of FWI historical hindcast is 5.1, which indicates a very low-level risk of forest fires for Region-4 concerning EFFIS FWI Classification. Moreover, the average values of FWI forecasts with RCP4.5 and RCP8.5 scenarios by MPI-ESM-MR are respectively 8.4 (low-level fire risk) and 13.2 (moderate-level fire risk) in the future. These average values of FWI forecasts demonstrate that in the future, forest fire risk will increase 65% under the low-concentration scenario RCP4.5 and 159% under the high-concentration scenario RCP8.5.

Moreover, according to the results of the HadGEM2-ES model, the average value of FWI re-forecast is 12.5, which shows a moderate-level risk of forest fires for Region-4. The average values of FWI outputs with RCP4.5 and RCP8.5 scenarios by HadGEM2-ES are respectively 21.2 (moderate-level fire risk) and 36.5 (high-level fire risk). These average values of FWI forecasts demonstrate that the risk of forest fires will raise 67% under the low-concentration scenario RCP4.5 and 192 % under the high-concentration scenario RCP8.5 in the future.

The results of FWI re-forecasts provide that fire risk in this region is very low in terms of the result of MPI-ESM-MR historical hindcast. However, it is moderate according to the outputs of HadGEM2-ES re-forecast. The MPI-ESM-MR model result gives a more accurate estimation than the output of the HadGEM2-ES model based on the re-forecast result of observational data (ERA-interim) (Figure 4.1). The result of the MPI-ESM-MR model demonstrates a significant increase in the forest fire risk in Emilia-Romagna and the southern Marche of Italy. Additionally, the FWI forecast of the HadGEM2-ES model shows a significant increase in fire danger, which is more than the prediction results of the MPI-ESM-MR model. All in all, forest fires start to be a factor that threatens forests in the Region-4.

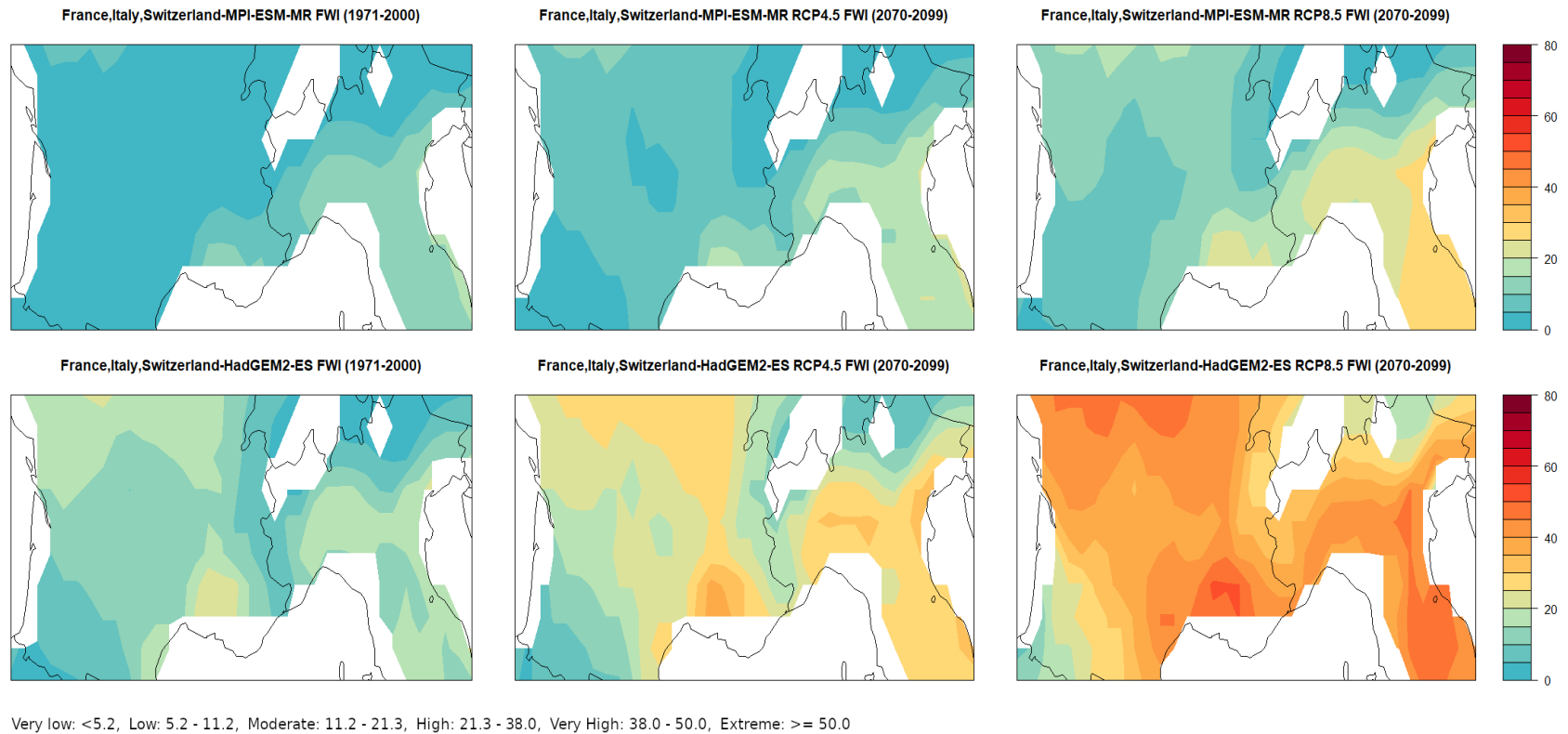


Figure 4.15. The Southern France, The Northern Italy, the Southern Switzerland- the historical hindcast FWI - HadGEM2-ES and MPI-ESM-MR (1971–2000) outputs and the outputs of FWI - MPI-ESM-MR and HadGEM2-ES models (2070–2099) with RCP4.5 and RCP8.5 emission scenarios

4.1.5. FWI Results of Region-5

In Figure 4.16, FWI outputs of re-forecasts and forecasts (RCP4.5, RCP8.5) by MPI-ESM-MR and HadGEM2-ES were given for Region-5, which surround the Iberian Peninsula. According to the results of the MPI-ESM-MR model, the average value of FWI historical hindcast is 13.4, which indicates a moderate-level risk of forest fires for Region-1 concerning EFFIS FWI Classification. Moreover, the average values of FWI forecasts with RCP4.5 and RCP8.5 scenarios by MPI-ESM-MR are respectively 18.3 (moderate-level fire risk) and 24.8 (high-level fire risk) in the future. These average values of FWI forecasts demonstrate that in the future, forest fire risk will increase 37% under the low-concentration scenario RCP4.5 and 85% under the high-concentration scenario RCP8.5.

Furthermore, according to the results of the HadGEM2-ES model, the average value of FWI re-forecast is 14.4, which shows a moderate-level risk of forest fires for Region-1. The average values of FWI outputs with RCP4.5 and RCP8.5 scenarios by HadGEM2-ES are respectively 23.6 (high-level fire risk) and 41.7 (very high-level fire risk). These average values of FWI forecasts demonstrate that the risk of forest fires will raise 64% under the low-concentration scenario RCP4.5 and 190 % under the high-concentration scenario RCP8.5 in the future (Figure 4.16).

The results of FWI re-forecasts provide that fire risk in this region is moderate in terms of the result of MPI-ESM-MR and HadGEM2-ES re-forecast. The MPI-ESM-MR model result gives a more accurate estimation than the output of the HadGEM2-ES model based on the re-forecast result of observational data (ERA-interim) (Figure 4.1). The risk of forest fires will extremely increase in the southern part of Spain (Andalusia) according to future forecasts. Additionally, the FWI forecast of the HadGEM2-ES model shows a significant increase in fire danger, which is more than the prediction results of the MPI-ESM-MR model. All in all, forest fires start to be a serious issue that threatens forests in the Region-5.

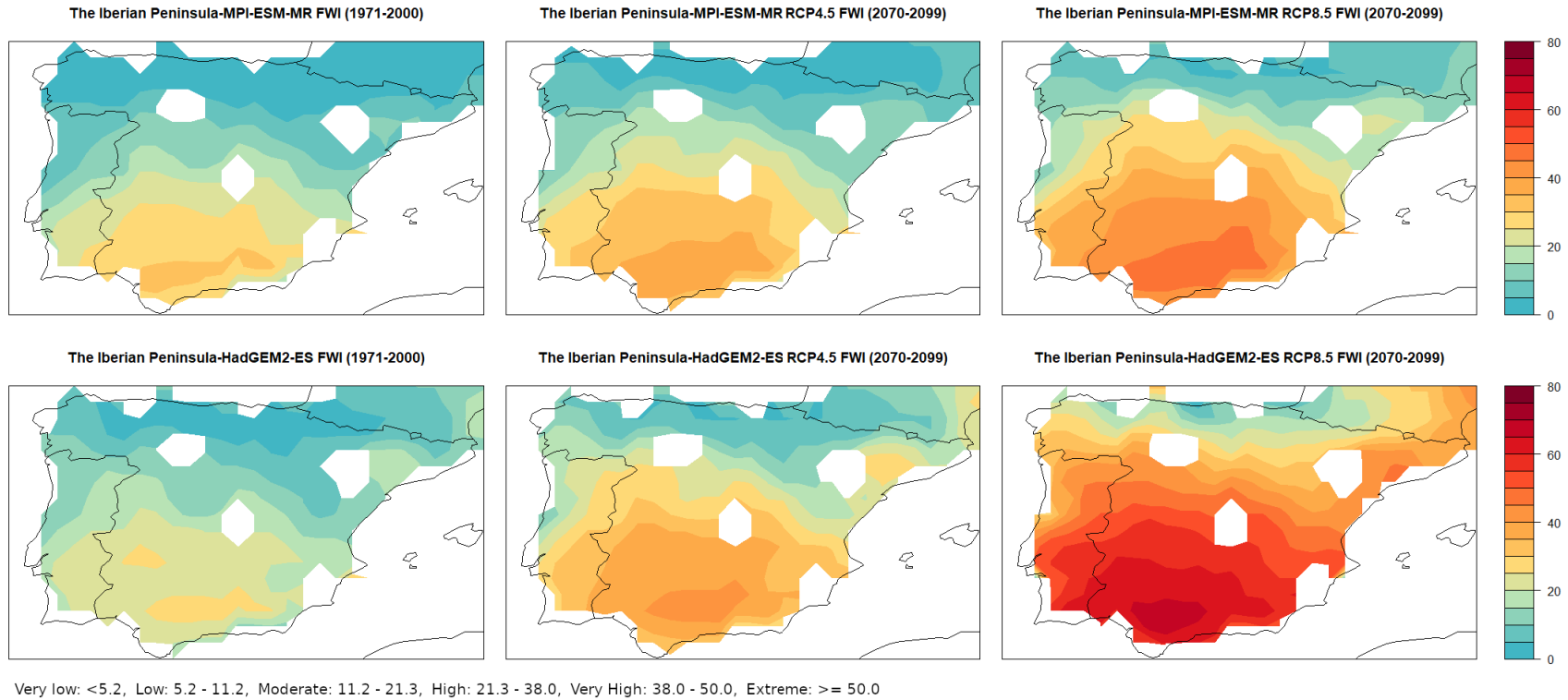


Figure 4.16. The Iberian Peninsula- the historical hindcast FWI - HadGEM2-ES and MPI-ESM-MR (1971–2000) outputs and the outputs of FWI - MPI-ESM-MR and HadGEM2-ES models (2070–2099) with RCP4.5 and RCP8.5 emission scenarios

4.2. Greenhouse Experiment

4.2.1. Metal Analysis of the Soil

Metal analysis of two different soil sample, one of soil sample taken from mine tailing area in Kütahya and other taken from the forest area in Muğla, were done by ICP-OES method before the greenhouse set up. The metals of Cr, Mn, Fe, Ni, Cu, Zn, Al, Cd, Pb, Si, Co and Mo concentrations in the soil were evaluated. In the mine soil iron and aluminum were found as the highest concentrations among these 12 metals (Table 4.1).

Table 4.1. The results of metals analysis in the mine soil.

Sample	Sample size (g)	Cr (ppm)	Mn (ppm)	Fe (ppm)	Ni (ppm)	Cu (ppm)	Zn (ppm)	Al (ppm)	Cd (ppm)	Pb (ppm)	Si (ppm)	Co (ppm)	Mo (ppm)
Sample-1	0.2573	4.35	7.27	703	9.94	0.336	0.160	52.6	0.002	0.029	3.62	0.359	0.008
Sample-2	0.2588	4.74	4.08	677	11.10	0.350	0.171	95.6	0.003	0.029	3.88	0.425	0.033
Sample-3	0.2560	4.80	3.45	609	9.35	0.298	0.129	80.5	0.003	0.025	3.87	0.342	0.009

Type of Muğla soil is red Mediterranean soil, also known as terra rossa. This type soil includes high amount of iron oxide and limestone (Durn, 2003). The result of metal analysis of Muğla soil includes high concentrations of iron and aluminum like mine soil from Kütahya (Table 4.2).

Table 4.2. The results of metals analysis in the Muğla soil

Sample	Sample size (g)	Cr (ppm)	Mn (ppm)	Fe (ppm)	Ni (ppm)	Cu (ppm)	Zn (ppm)	Al (ppm)	Cd (ppm)	Pb (ppm)	Si (ppm)	Co (ppm)	Mo (ppm)
Sample-1	0.2545	2.022	0.633	338.507	1.902	0.028	0.200	63.517	0.000	0.007	0.223	0.078	0.001
Sample-2	0.2545	1.041	0.344	244.597	1.118	0.019	0.040	43.910	0.000	0.006	0.178	0.055	0.000

4.2.2. Greenhouse Set Up

At the end of the study, the difference between saplings of AMF (+) pots and AMF (-) (control group) pots were observed clearly since the presence of AMF promotes a high growth rate in AMF (+) pots. Moreover, the control group (AMF (-)) of *Pinus nigra* saplings in Muğla soil dried, but AMF (+) group saplings grew up well during the experiment (Figure 4.17).

Although one sapling in the pot of Muğla soil-*Pinus nigra* AMF (-) (control group) was changed with new sapling (1) at 2nd week of the experiment after dried (left side in the AMF (-) pot), sapling-1 dried at 9th week and sapling-2 dried just after the end of experiment.



Figure 4.17. Dried saplings in Muğla soil- *Pinus nigra* AMF (-) pot (left side) and saplings in Muğla soil- *Pinus nigra* AMF (+) pot (right side)

In the Muğla soil- *Quercus petraea* (oak) pots there was no big difference between the growth rate of saplings in AMF (+) pots and in AMF (-) (control group) pots (Figure 4.18). However, saplings in the control group pot were affected by powdery mildew disease which is a fungal disease (Figure 4.19). White powdery spots on the leaves were observed first in June and there were not used any chemicals to controlled disease. Only, infected leaves were cut, but it did not prevent to spread the disease.

On the other hand, saplings in AMF (+) pots were not infected by powdery mildew disease even though saplings were closer to infected plants. AMF is involved in protection against root pathogens (Rillig, 2004). Oak saplings in AMF (+) pots would not be affected powdery mildew since the soil in this pot included both Fe and AMF. Fe has a special role in the formation of glomalin, which is a protein produced by AMF. Fe in the organic matter could provide the thermal stability and antimicrobial properties of glomalin (Nichols et al. 2005; Pei et al, 2019).



Figure 4.18. Saplings in Muğla soil- *Quercus petraea* (oak) AMF (-) pot (left side) and saplings in Muğla soil- *Quercus petraea* AMF (+) pot (right side)



Figure 4.19. Infected sapling in Muğla soil- *Quercus petraea* (oak) AMF (-) pot (left side) and sapling not infected in Muğla soil- *Quercus petraea* AMF (+) pot (right side)

Oak (*Quercus robur*) saplings in mine soil AMF (+) and AMF (-) pots were planted in the 2nd week of the experiment since previous oak saplings (*Quercus petraea*) planted in pots dried. (Figure 4.20), so the measurements of soil moisture of these pots were started in the 2nd week.

The difference between the measurements of soil moisture in the pot of *Quercus robur* AMF (+)-mine soil and the *Quercus robur* AMF(-) mine soil pot was high under the water stress since AMF decrease drought stress in their host plants by increasing uptake and transfer of water via the fungal hyphae to the host plants (Khalvati et al., 2005). During the study, all *Quercus robur* saplings were well but one sapling in the control group pot dried at the end of the study (Figure 4.20).



Figure 4.20. Saplings in mine soil- *Quercus robur* (oak) AMF (-) pot (left side) and saplings in mine soil- *Quercus robur* AMF (+) pot (right side)

AMF provides improvement of water and nutrient uptake and drought tolerance for host plants (Rillig, 2004). The development of plant water relations and biomass production were clearly observed in mine soil-*Pinus nigra* AMF (+) pot in the greenhouse study (Figure 4.21).

Moreover, saplings in both AMF (+) mine soil pot and AMF (-) mines soil pot grew up efficiently during the study. On the other hand, saplings in AMF (+) pot grew up more than saplings in another group. The difference between saplings in the two groups can be seen in Figure 4.21. It would be related to soil taken from coal mine since it included high amount of carbon and enough concentration of micronutrients of manganese (Mn), iron (Fe), copper (Cu), Zinc (Zn), molybdenum (Mo), which

are essential elements for plant growth (Harmsen, et al. 1985). it may have a positive impact on saplings.



Figure 4.21. Saplings in mine soil- *Pinus nigra* AMF (-) pot (left side) and saplings in mine soil- *Pinus nigra* AMF (+) pot (right side)

4.2.3. Soil Moisture, pH & Salt Activity Measurements

Soil moisture, temperature, pH and salt content of the samples were quantified using COMBI 5000 twice a week throughout the greenhouse study. Measurements of both soil moisture and temperature in pots were done by near two saplings in each pot.

Measurements of salt content (AM) in Muğla soil- *Quercus petraea* control group (AMF(-)) pot demonstrated that the average salt content of the soil was 0.11 g/L (± 0.075 g/L). Additionally, the temperature was usually higher than 25 °C during the study. Soil pH in Muğla soil- *Quercus petraea* AMF (-) pot was measured 6.72 which is fine for plant growth since when soil pH is 6.5–7.5 (near neutral), most mineral nutrients are readily available to plants (Gould and Walker, 1999; Soti et al., 2015). Additionally, the same amount of water was given for all saplings throughout the greenhouse study. Soil moisture measurements demonstrated that moisture inside Muğla soil- *Quercus petraea* (oak) AMF (-) pot was adjusted to lower than 20 % (Table 4.3).

Table 4.3. Measurements of soil moisture, external temperature, pH and salt content in Muğla soil- AMF (-) oak pot

DATE	MUĞLA SOIL- <i>Quercus petraea</i> AMF (-)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	Sapling-1		Sapling-2		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
24.07.2018	26.10	26.30	26.20	6.36	NA	6.34	NA	6.35	6.69	6.73	6.71	NA	NA	NA
27.07.2018	30.00	28.80	29.90	12.30	NA	12.70	NA	12.50	6.96	6.94	6.95	0.10	0.09	0.10
31.07.2018	30.80	31.00	30.90	15.60	NA	15.40	NA	15.50	7.20	7.56	7.38	0.10	0.10	0.10
03.08.2018	30.90	30.90	31.90	14.32	NA	14.38	NA	14.35	7.20	7.32	7.26	0.12	0.16	0.14
07.08.2018	30.20	30.40	30.30	12.79	NA	12.91	NA	12.85	6.99	6.93	6.96	0.12	0.10	0.11
10.08.2018	30.90	31.00	30.95	15.60	NA	15.26	NA	15.43	6.61	6.93	6.77	0.13	0.18	0.16
14.08.2018	32.10	32.40	32.25	12.97	NA	15.20	NA	14.09	6.64	6.51	6.58	0.08	0.13	0.11
17.08.2018	31.70	31.80	31.75	11.14	NA	12.64	NA	11.89	6.77	6.59	6.68	0.09	0.08	0.09
20.08.2018	30.20	30.30	30.25	11.65	NA	15.32	NA	13.49	6.85	6.83	6.84	0.09	0.11	0.10
24.08.2018	30.00	29.90	29.95	10.13	8.30	9.50	9.63	9.39	6.88	6.54	6.71	0.09	0.08	0.09
27.08.2018	30.70	30.30	30.50	9.34	7.10	8.75	7.34	8.13	6.47	6.28	6.38	0.06	0.07	0.07
31.08.2018	32.60	32.10	32.35	9.63	6.10	8.96	6.75	7.86	7.18	7.09	7.14	0.45	0.24	0.35
04.09.2018	31.70	31.60	31.65	8.07	6.64	9.20	6.40	7.58	6.97	7.15	7.06	0.08	0.09	0.09
08.09.2018	28.30	28.00	28.15	13.63	12.68	12.32	9.75	12.10	6.50	6.47	6.49	0.09	0.12	0.11
11.09.2018	25.80	24.50	25.15	11.60	10.02	8.30	10.25	10.04	6.65	6.56	6.61	0.11	0.10	0.11
13.09.2018	30.40	30.20	30.30	11.28	9.34	9.62	8.15	9.60	6.65	6.70	6.68	0.08	0.10	0.09
17.09.2018	28.40	27.60	28.00	11.45	12.95	12.10	9.31	11.45	6.81	6.95	6.88	0.10	0.09	0.10
21.09.2018	29.70	29.20	29.45	11.53	9.30	7.80	7.40	9.01	6.54	6.65	6.60	0.08	0.11	0.10
25.09.2018	23.20	24.00	23.60	10.91	12.55	8.06	7.51	9.76	6.84	6.78	6.81	0.10	0.10	0.10
28.09.2018	27.40	28.70	28.05	10.30	9.02	6.51	7.28	8.28	6.89	6.89	6.89	0.09	0.09	0.09
01.10.2018	29.10	29.50	29.30	10.30	10.02	8.35	9.68	9.59	6.78	6.81	6.80	0.09	0.10	0.10
05.10.2018	27.50	27.80	27.65	11.70	10.68	13.80	11.13	11.83	6.81	6.92	6.87	0.08	0.10	0.09
08.10.2018	27.20	27.60	27.40	9.21	10.54	10.40	7.95	9.53	6.59	6.61	6.60	0.10	0.10	0.10
12.10.2018	29.70	29.90	29.80	11.73	9.95	11.03	8.00	10.18	6.41	6.60	6.51	0.11	0.09	0.10
15.10.2018	24.90	25.70	25.30	8.15	11.25	7.71	10.81	9.48	6.74	6.80	6.77	0.11	0.10	0.11
19.10.2018	27.40	28.10	27.75	7.85	9.19	6.71	6.84	7.65	6.44	6.63	6.54	0.08	0.09	0.09

M1: 1st measurement, M2: 2nd measurement

Measurements of soil moisture, temperature, pH, and salt content in Muğla soil- *Quercus petrea* (oak) AMF (+) pot were given in Table 4.4. According to the results of measurements, the average salt content of the soil was 0.10g/L (± 0.075 g/L).

The average value of pH measurements demonstrated that soil pH was 6.85 in Muğla soil- *Quercus petrea* AMF (+) pot. It is an appropriate value for plant growth because nutrients are more readily available to plants when soil pH is between 6.5 and 7.5. Moreover, root colonization by mycorrhizal fungi was higher in soil pH 5.5-7.5 (Pausas and Austin, 2001; Soti et al., 2015), which affects directly water and nutrient uptake to host plants.

Furthermore, according to Table 4.4, soil moisture inside Muğla soil- *Quercus petrea* AMF (+) pot was adjusted to lower than 17%. This situation indicated that saplings were under water stress throughout the greenhouse study. Moreover, the plants were grown for three months under higher temperatures (avg. max temperature: 30.43 °C and avg. min temperature: 22.18 °C) in the greenhouse and the consequence of the study showed that under drought conditions and high temperature, AMF improves plant growth and symbiotic relations. On the other hand, one oak sapling in the AMF (+) pot dried in the 9th week of the study. It may be related to failed AMF inoculation to this sapling.

According to the comparison of soil moisture content between AMF (+) and AMF (-) pots of Muğla soil- *Quercus petrea*, soil moisture content of AMF (+) pot was quantified higher than AMF (-) pot (Figure 4.22) even though all pots watered with the same amount of water during the study. It showed that mycorrhizal fungi are capable of directly mobilizing water to plants.

Table 4.4. Measurements of soil moisture, external temperature, pH and salt content in Muğla soil- AMF (+) oak pot

DATE	MUĞLA SOIL- <i>Quercus petraea</i> AMF (+)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
24.07.2018	26.10	26.10	26.10	6.50	NA	6.80	NA	6.65	6.80	6.94	6.87	NA	NA	NA
27.07.2018	29.40	29.60	29.50	13.00	NA	15.00	NA	14.00	7.40	7.42	7.41	0.14	0.12	0.13
31.07.2018	31.90	31.70	31.80	16.00	NA	17.00	NA	16.50	7.10	7.14	7.12	0.11	0.10	0.11
03.08.2018	31.80	31.60	31.70	15.00	NA	15.24	NA	15.12	7.40	7.00	7.20	0.15	0.17	0.16
07.08.2018	32.00	32.00	32.00	14.00	NA	13.30	NA	13.65	7.12	7.30	7.22	0.16	0.16	0.16
10.08.2018	31.20	31.40	31.30	14.86	NA	17.45	NA	16.16	6.99	6.88	6.94	0.10	0.11	0.11
14.08.2018	32.10	32.20	32.15	13.71	NA	11.23	NA	12.47	6.72	6.80	6.76	0.11	0.08	0.10
17.08.2018	32.00	32.00	32.00	10.45	NA	11.85	NA	11.15	6.80	6.80	6.80	0.13	0.09	0.11
20.08.2018	31.40	31.20	31.30	11.40	NA	16.00	NA	13.70	6.93	6.96	6.95	0.13	0.12	0.13
24.08.2018	30.50	30.00	30.25	10.70	8.11	12.90	11.25	10.74	6.88	6.93	6.91	0.10	0.10	0.10
27.08.2018	31.30	30.50	30.90	8.46	8.40	9.50	11.80	9.54	6.44	6.50	6.47	0.09	0.10	0.10
31.08.2018	32.80	32.80	32.80	8.05	8.23	11.92	8.85	9.26	6.86	7.12	6.99	0.08	0.14	0.11
04.09.2018	30.70	30.40	30.55	8.12	8.78	9.02	8.35	8.57	7.12	7.18	7.15	0.08	0.08	0.08
08.09.2018	28.90	28.90	28.90	12.70	11.75	16.63	13.44	13.63	6.67	6.60	6.64	0.10	0.12	0.11
11.09.2018	24.80	24.70	24.75	11.80	10.45	13.66	11.53	11.86	6.95	6.65	6.80	0.10	0.10	0.10
13.09.2018	31.20	31.00	31.10	11.56	8.96	10.15	11.38	10.51	6.81	6.84	6.83	0.10	0.14	0.12
17.09.2018	29.30	29.40	29.35	11.08	12.09	14.34	12.73	12.56	6.89	6.92	6.91	0.11	0.09	0.10
21.09.2018	30.10	30.00	30.05	10.17	7.71	9.80	11.00	9.67	6.95	6.97	6.96	0.10	0.08	0.09
25.09.2018	24.40	24.30	24.35	10.60	7.45	NA	NA	9.03	7.00	NA	7.00	0.08	NA	0.08
28.09.2018	29.40	30.10	29.75	10.85	7.30	NA	NA	9.08	6.81	NA	6.81	0.11	NA	0.11
01.10.2018	30.20	30.60	30.40	11.30	8.10	NA	NA	9.70	7.00	NA	7.00	0.10	NA	0.10
05.10.2018	28.10	28.60	28.35	12.10	10.05	NA	NA	11.08	6.97	NA	6.97	0.13	NA	0.13
08.10.2018	28.10	28.00	28.05	10.70	9.67	NA	NA	10.19	6.73	NA	6.73	0.10	NA	0.10
12.10.2018	30.00	30.00	30.00	12.28	10.01	NA	NA	11.15	6.60	NA	6.60	0.10	NA	0.10
15.10.2018	26.40	27.00	26.70	11.85	10.50	NA	NA	11.18	6.88	NA	6.88	0.10	NA	0.10
19.10.2018	26.90	28.30	27.60	10.63	8.47	NA	NA	9.55	6.69	NA	6.69	0.09	NA	0.09

M1: 1st measurement, M2: 2nd measurement

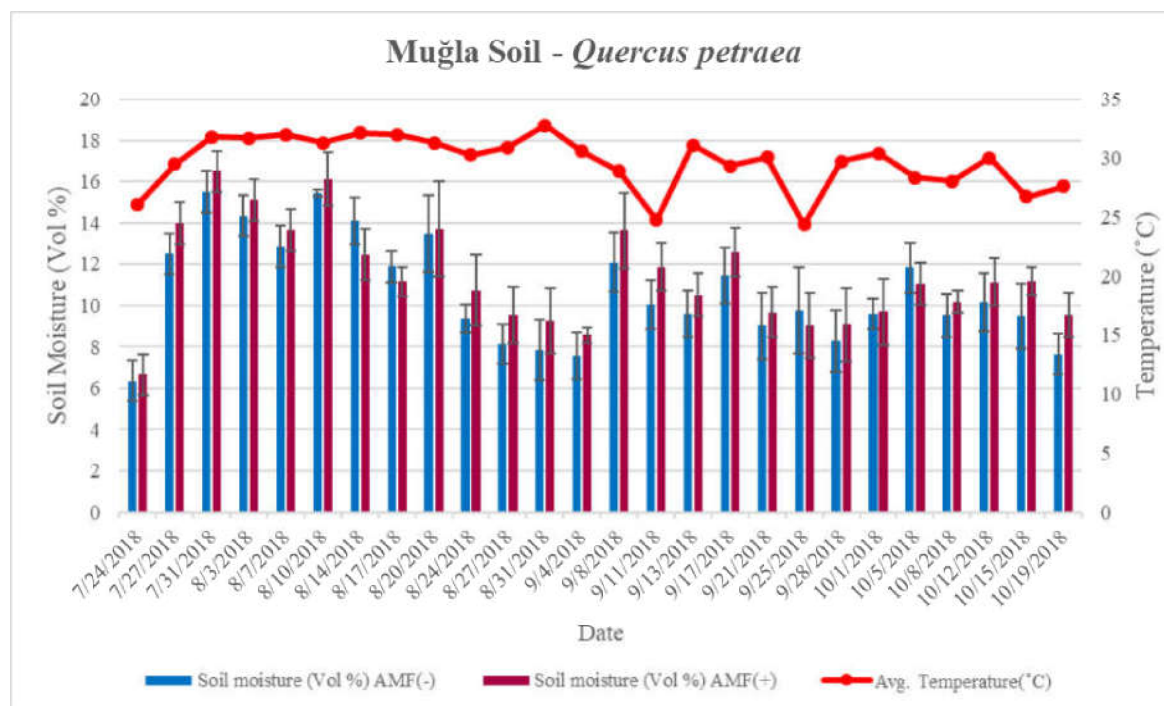


Figure 4.22. The comparison of soil moisture content between Muğla soil- AMF (+) oak pot and Muğla soil- AMF (-) oak pot

Measurements of salt content (AM) in Muğla soil-*Pinus nigra* AMF (-) (control group) pot demonstrated that the average salt content of the soil was 0.10 g/L (± 0.075 g/L) (Table 4.5). Moreover, the greenhouse temperature was usually higher than 25 °C (avg. max temp 30 °C) during the study. Soil pH in Muğla soil- *Pinus nigra* AMF (-) pot was measured 6.55, which indicated soil pH was near neutral. It is a suitable value for plant growth due to many available mineral nutrients to plants. Additionally, the same amount of water was given for all saplings throughout the greenhouse study. Soil moisture measurements demonstrated that moisture inside Muğla soil- *Pinus nigra* AMF (-) pot was adjusted to lower than 20 % (Table 4.5).

Table 4.5. Measurements of soil moisture, external temperature, pH and salt content in Muğla soil- AMF (-) *Pinus nigra* pot

DATE	MUĞLA SOIL- <i>Pinus nigra</i> AMF (-)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
			M1	M2	M1	M2								
24.07.2018	24.60	24.20	24.40	3.40	NA	4.20	NA	3.80	6.80	6.40	6.60	NA	NA	NA
27.07.2018	29.30	29.90	29.60	13.60	NA	13.76	NA	13.68	6.50	6.40	6.45	0.24	0.20	0.22
31.07.2018	30.50	30.90	30.70	21.18	NA	23.14	NA	22.16	7.00	7.36	7.18	0.22	0.22	0.22
03.08.2018	30.80	31.00	30.90	19.20	NA	19.30	NA	19.25	6.44	6.60	6.52	0.30	0.32	0.32
07.08.2018	29.80	29.40	29.60	19.50	NA	21.00	NA	20.25	6.90	6.80	6.85	0.20	0.22	0.21
10.08.2018	31.00	30.90	30.95	13.15	NA	11.80	NA	12.48	6.17	6.03	6.10	0.10	0.11	0.11
14.08.2018	32.00	32.40	32.20	9.87	NA	11.67	NA	10.77	6.43	6.22	6.33	0.11	0.11	0.11
17.08.2018	29.50	29.00	29.25	9.15	NA	11.00	NA	10.08	6.30	6.51	6.41	0.12	0.08	0.10
20.08.2018	29.80	30.10	29.95	10.45	NA	13.19	NA	11.82	6.16	6.75	6.46	0.10	0.11	0.11
24.08.2018	29.00	29.00	29.00	8.66	6.98	9.85	8.09	8.40	6.54	6.40	6.47	0.09	0.09	0.09
27.08.2018	29.70	29.20	29.45	8.40	7.30	8.99	7.02	7.93	6.14	6.03	6.09	0.10	0.08	0.09
31.08.2018	31.30	30.90	31.10	8.90	8.18	8.63	6.30	8.00	6.50	6.33	6.42	0.11	0.08	0.10
04.09.2018	31.40	31.50	31.45	7.52	6.27	8.55	7.15	7.37	6.64	6.94	6.79	0.08	0.13	0.11
08.09.2018	27.20	26.80	27.00	12.85	12.56	12.91	12.60	12.73	6.39	6.42	6.41	0.11	0.14	0.13
11.09.2018	24.30	24.40	24.35	9.91	7.05	10.13	7.58	8.67	6.54	6.81	6.68	0.12	0.12	0.12
13.09.2018	29.10	28.30	28.70	6.69	8.50	8.30	7.76	7.81	6.62	6.59	6.61	0.13	0.12	0.13
17.09.2018	26.90	27.30	27.10	12.28	8.78	NA	NA	10.53	6.70	NA	6.70	0.13	NA	0.13
21.09.2018	28.10	28.40	28.25	9.30	7.36	NA	NA	8.33	6.78	NA	6.78	0.12	NA	0.12
25.09.2018	21.80	21.80	21.80	9.70	8.63	NA	NA	9.17	6.89	NA	6.89	0.09	NA	0.09
28.09.2018	26.50	26.40	26.45	8.10	7.45	NA	NA	7.78	6.76	NA	6.76	0.08	NA	0.08
01.10.2018	27.30	27.60	27.45	10.58	9.45	NA	NA	10.02	6.70	NA	6.70	0.10	NA	0.10
05.10.2018	27.10	27.10	27.10	10.06	10.85	NA	NA	10.46	6.92	NA	6.92	0.08	NA	0.08
08.10.2018	26.00	26.00	26.00	12.50	9.65	NA	NA	11.08	6.59	NA	6.59	0.11	NA	0.11
12.10.2018	26.10	27.00	26.55	11.96	9.40	NA	NA	10.68	6.55	NA	6.55	0.09	NA	0.09
15.10.2018	21.80	23.00	22.40	11.32	9.63	NA	NA	10.48	6.52	NA	6.52	0.08	NA	0.08
19.10.2018	25.90	25.90	25.90	10.17	8.82	NA	NA	9.50	6.41	NA	6.41	0.09	NA	0.09

M1: 1st measurement, M2: 2nd measurement

Evaluations of soil moisture, temperature, pH and salt content in Muğla soil- *Pinus nigra* AMF (+) pot were given in Table 4.6. The average salt content of the soil was 0.10g/L (± 0.075 g/L). It was similar to Muğla soil AMF (-) *Pinus nigra* pot.

Temperature measured was usually higher than 25 °C when soil moisture evaluation was done. Soil pH was 6.74 in Muğla soil- *Pinus nigra* AMF (+) pot that provides a suitable area for plant growth because soil pH influences plant growth, nutrient availability and root colonization by mycorrhizal fungi (Table 4.7).

Additionally, according to Table 4.6, soil moisture inside Muğla soil- *Pinus nigra* AMF (+) pot was adjusted to lower than 15%. This situation demonstrated that saplings were under water stress during the greenhouse study. Moreover, the plants were grown for three months under higher temperatures in the greenhouse and the result of the study showed that under water stress and high temperature, AMF enhances plant growth and symbiotic relations.

Figure-4.23 provides the comparison of soil moisture content between pots of Muğla soil- *Pinus nigra* AMF (+) and AMF (-). The consequences indicated that soil moisture content of AMF (+) pot was usually lower than soil moisture in AMF (-) pot. This situation could be related to saplings in AMF (+) pot grew up more than other saplings in AMF (-) pot and then could start to use more water than other control group saplings. At the same time, the same amount of water was given for all pots during the experiment, but one sapling in the Muğla soil- *Pinus nigra* AMF (-) pot dried in the 9th week (Table 4.6) and this may be affected results.

Table 4.6. Measurements of soil moisture, external temperature, pH and salt content in Muğla soil- AMF (+) *Pinus nigra* pot

DATE	MUĞLA SOIL- <i>Pinus nigra</i> AMF (+)													
	Temp (°C)			Soil moisture (Vol %)				pH			AM (g/L)			
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
			M1	M2	M1	M2								
24.07.2018	25.00	24.80	24.90	5.00	NA	5.06	NA	5.03	6.16	7,20	6.68	NA	NA	NA
27.07.2018	29.60	29.00	29.30	15.08	NA	14.08	NA	14.58	7.22	7.30	7.26	0.12	0.14	0.13
31.07.2018	30.60	31.00	30.80	18.30	NA	19.00	NA	18.65	7.25	7.21	7.23	0.19	0.19	0.19
03.08.2018	31.60	31.00	31.30	16.50	NA	16.00	NA	16.25	7.40	7.24	7.32	0.14	0.12	0.13
07.08.2018	30.90	30.70	30.80	13.00	NA	11.70	NA	12.35	7.18	7.14	7.16	0.11	0.11	0.11
10.08.2018	31.20	31.30	31.25	13.50	NA	11.84	NA	12.67	7.09	7.07	7.08	0.09	0.09	0.09
14.08.2018	32.40	33.00	32.70	10.20	NA	8.63	NA	9.42	6.59	6.80	6.70	0.10	0.09	0.10
17.08.2018	31.60	31.30	31.45	8.21	NA	9.55	NA	8.88	6.67	6.54	6.61	0.09	0.08	0.09
20.08.2018	30.40	30.80	30.60	8.35	NA	5.18	NA	6.77	6.93	6.72	6.83	0.14	0.10	0.12
24.08.2018	31.60	31.40	31.50	7.04	6.35	6.20	8.30	6.97	6.59	6.67	6.63	0.10	0.11	0.11
27.08.2018	33.20	32.70	32.95	5.55	6.71	5.93	6.14	6.08	6.35	6.42	6.39	0.09	0.08	0.09
31.08.2018	31.60	32.60	32.10	7.00	7.29	6.97	8.10	7.34	6.92	6.94	6.93	0.09	0.11	0.10
04.09.2018	32.10	31.50	31.80	7.73	6.60	6.01	9.12	7.37	6.89	7.00	6.95	0.06	0.09	0.08
08.09.2018	27.30	27.40	27.35	10.77	9.45	11.45	12.91	11.15	6.44	6.50	6.47	0.12	0.09	0.11
11.09.2018	25.30	25.20	25.25	11.44	9.05	9.95	9.30	9.94	6.81	6.65	6.73	0.11	0.09	0.10
13.09.2018	31.50	31.80	31.65	9.02	8.35	10.00	9.26	9.16	6.73	6.70	6.72	0.10	0.09	0.10
17.09.2018	29.70	29.50	29.60	9.56	8.00	9.47	8.40	8.86	6.81	6.97	6.89	0.10	0.10	0.10
21.09.2018	30.30	30.40	30.35	8.93	10.25	8.13	8.70	9.00	6.59	6.67	6.63	0.09	0.08	0.09
25.09.2018	25.60	26.50	26.05	10.00	9.10	8.50	10.12	9.43	6.89	6.92	6.91	0.09	0.10	0.10
28.09.2018	30.10	30.70	30.40	8.15	9.15	8.38	7.50	8.30	6.57	6.85	6.71	0.09	0.09	0.09
01.10.2018	31.00	30.80	30.90	8.65	10.75	8.35	9.68	9.36	6.86	6.84	6.85	0.10	0.12	0.11
05.10.2018	28.50	28.70	28.60	11.34	10.25	14.52	14.26	12.59	7.06	7.06	7.06	0.13	0.09	0.11
08.10.2018	30.60	29.50	30.05	11.75	11.20	10.05	8.80	10.45	6.73	6.70	6.72	0.10	0.08	0.09
12.10.2018	30.40	30.10	30.25	12.05	10.20	7.75	9.75	9.94	6.52	6.58	6.55	0.10	0.10	0.10
15.10.2018	29.80	28.70	29.25	10.05	8.25	8.25	8.75	8.83	6.58	6.60	6.59	0.09	0.10	0.10
19.10.2018	29.60	29.30	29.45	8.71	9.12	10.68	7.87	9.10	6.50	6.55	6.53	0.08	0.08	0.08

M1: 1st measurement, M2: 2nd measurement

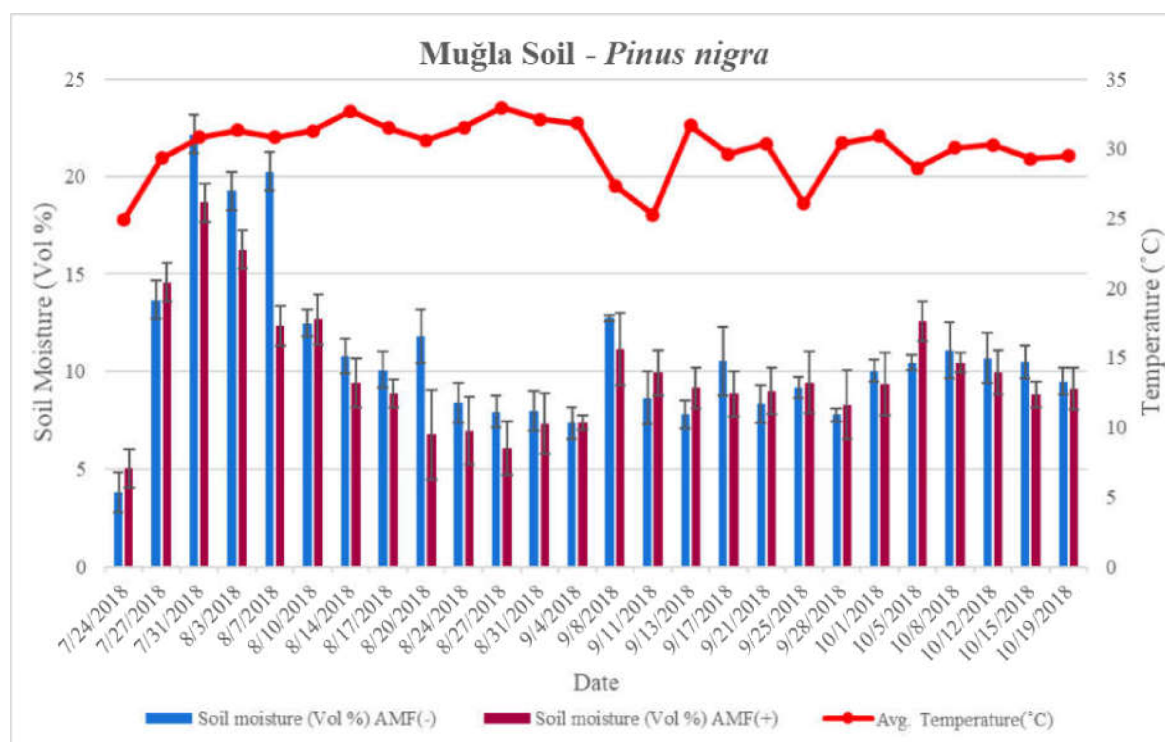


Figure 4.23. The comparison of soil moisture content between Muğla soil- AMF (+) *Pinus nigra* pot and Muğla soil- AMF (-) *Pinus nigra* pot

Measurements of salt content (AM) in mine soil- *Quercus robur* AMF (-) pot showed that the average salt content of the soil was 0.28g/L (± 0.075 g/L) (Table 4.8). Moreover, the greenhouse temperature was usually higher than 25 °C (avg. max temp 30 °C) during the study.

Soil pH in mine soil- *Quercus robur* AMF (-) pot was measured 6.86, which indicated soil pH was near neutral, so it is a suitable value for plant growth. Additionally, the same amount of water was given for all saplings throughout the greenhouse study. Soil moisture measurements demonstrated that moisture inside mine soil- *Quercus robur* AMF (-) pot was adjusted to lower than 20 % (Table 4.7).

Table 4.7. Measurements of soil moisture, external temperature, pH and salt content in mine soil- AMF (-) *Quercus robur* pot

DATE	MINE SOIL- <i>Quercus robur</i> AMF (-)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
31.07.2018	31.50	31.30	31.40	21.60	NA	21.70	NA	21.65	7.20	7.26	7.23	0.61	0.65	0.63
03.08.2018	33.00	33.40	33.20	19.90	NA	19.80	NA	19.85	6.91	6.93	6.92	0.49	0.51	0.50
07.08.2018	32.70	32.30	32.50	15.50	NA	14.14	NA	14.82	6.92	6.94	6.93	0.41	0.41	0.41
10.08.2018	32.20	32.20	32.20	10.34	NA	11.15	NA	10.75	6.50	6.77	6.64	0.18	0.10	0.14
14.08.2018	32.30	32.00	32.15	8.35	NA	8.65	NA	8.50	6.64	6.80	6.72	0.20	0.10	0.15
17.08.2018	32.30	32.10	32.20	13.58	NA	11.85	NA	12.72	6.85	6.91	6.88	0.18	0.19	0.19
20.08.2018	31.00	30.80	30.90	11.16	NA	11.62	NA	11.39	6.85	6.85	6.85	0.27	0.28	0.28
24.08.2018	30.40	30.50	30.45	7.75	10.38	9.33	11.00	9.62	6.88	6.83	6.86	0.20	0.25	0.23
27.08.2018	32.30	32.10	32.20	10.90	13.20	10.63	8.76	10.87	6.67	6.78	6.73	0.16	0.28	0.22
31.08.2018	31.40	31.50	31.45	12.35	11.58	12.47	10.46	11.72	7.18	7.09	7.14	0.45	0.24	0.35
04.09.2018	32.30	32.30	32.30	12.06	14.64	10.42	9.98	11.78	7.00	7.12	7.06	0.30	0.39	0.35
08.09.2018	27.40	28.30	27.85	15.25	14.60	14.13	11.95	13.98	6.92	7.03	6.98	0.29	0.30	0.30
11.09.2018	25.80	25.60	25.70	11.95	10.21	11.20	10.35	10.93	6.95	7.00	6.98	0.29	0.22	0.26
13.09.2018	31.50	31.00	31.25	12.30	9.26	10.67	9.60	10.46	6.83	6.92	6.88	0.44	0.22	0.33
17.09.2018	29.30	29.50	29.40	10.00	15.26	13.89	11.90	12.76	7.03	7.06	7.05	0.32	0.27	0.30
21.09.2018	30.30	30.60	30.45	12.50	12.40	9.70	11.20	11.45	6.81	6.75	6.78	0.29	0.38	0.34
25.09.2018	26.30	26.40	26.35	14.06	13.25	12.48	11.77	12.89	7.03	7.06	7.05	0.27	0.20	0.24
28.09.2018	31.10	30.90	31.00	12.90	9.18	10.38	9.40	10.47	6.70	6.86	6.78	0.43	0.30	0.37
01.10.2018	30.80	30.80	30.80	13.55	10.05	12.00	10.03	11.41	7.09	6.97	7.03	0.31	0.22	0.27
05.10.2018	28.10	28.30	28.20	15.07	14.81	18.88	15.13	15.97	6.97	7.00	6.99	0.40	0.30	0.35
08.10.2018	30.00	29.80	29.90	14.25	10.45	14.41	11.24	12.59	6.70	6.65	6.68	0.31	0.37	0.34
12.10.2018	29.30	29.50	29.40	11.07	16.47	15.76	12.91	14.05	6.74	6.77	6.76	0.23	0.38	0.31
15.10.2018	29.00	29.00	29.00	12.70	13.35	13.62	11.70	12.84	6.52	6.66	6.59	0.31	0.32	0.32
19.10.2018	28.90	29.00	28.95	13.04	12.84	14.37	10.86	12.78	6.75	6.66	6.71	0.26	0.32	0.29

M1: 1st measurement, M2: 2nd measurement

Evaluations of soil moisture, temperature, pH, and salt content in mine soil- *Quercus robur* AMF (+) pot was given in Table 4.8. The average mine soil salinity is 0.31 g/L (± 0.075 g/L), which indicated mine soil salinity in the AMF (+) pot was higher than mine soil salinity in the AMF (-) pot (0.28 g/L). Also, the salt content in the mine soil was higher than in Muğla soil. The temperature of the greenhouse was measured higher than 25 °C when evaluating soil moisture.

Soil pH in mine soil- *Quercus robur* AMF (+) pot was measured 6.96, which was higher than mine soil pH in AMF (-) *Quercus robur* pot, but it was fine for plant growth due to near neutral. Measured soil moisture values demonstrated that the soil moisture in mine soil- *Quercus robur* AMF (+) pot was adjusted to lower than 20 % (Table 4.8).

Figure-4.24 shows the comparison of soil moisture content between pots of mine soil- *Quercus robur* AMF (+) and AMF (-). The results of the study indicated that soil moisture content of AMF (+) pot was usually higher than soil moisture in AMF (-) pot, although two pots were in the same conditions. According to the study, mycorrhizal inoculation increased water uptake to the plant in mine soil- *Quercus robur* AMF (+) pot under high temperature and water stress throughout the greenhouse study. The difference in soil moisture between mycorrhizal and non-mycorrhizal group can be seen (Figure 4.24).

Moreover, drought is one of the significant problems reducing plant growth in the world (Auge, 2001; Khalvati et al., 2005). The result of the greenhouse study verified AMF enhances plant growth, symbiotic relations, and drought tolerance in the host plants. Thus, AMF can be inoculated not only in the forest area to increase or protect soil moisture but also, in the mining region for reforestation.

Table 4.8. Measurements of soil moisture, external temperature, pH and salt content in mine soil- AMF (+) *Quercus robur* pot

DATE	MINE SOIL- <i>Quercus robur</i> AMF (+)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
31.07.2018	31.70	32.10	31.90	22.98	NA	22.94	NA	22.96	7.48	7.40	7.44	0.82	0.80	0.81
03.08.2018	32.30	32.50	32.40	20.08	NA	18.50	NA	19.29	7.43	7.27	7.35	0.72	0.76	0.74
07.08.2018	32.10	32.30	32.20	16.20	NA	15.50	NA	15.85	7.36	7.20	7.28	0.60	0.78	0.69
10.08.2018	31.70	31.80	31.75	14.51	NA	12.80	NA	13.66	6.88	6.74	6.81	0.19	0.21	0.20
14.08.2018	32.10	32.00	32.05	11.47	NA	10.35	NA	10.91	6.88	6.85	6.87	0.12	0.21	0.17
17.08.2018	31.00	30.30	30.65	15.05	NA	13.50	NA	14.28	7.01	7.01	7.01	0.21	0.27	0.24
20.08.2018	30.60	30.60	30.60	12.65	NA	13.20	NA	12.93	6.96	7.01	6.99	0.42	0.49	0.46
24.08.2018	30.30	30.30	30.30	13.56	11.29	13.59	11.58	12.51	6.85	6.85	6.85	0.24	0.25	0.25
27.08.2018	32.40	32.00	32.20	11.28	12.83	13.97	10.84	12.23	6.97	6.89	6.93	0.14	0.30	0.22
31.08.2018	32.10	32.30	32.20	13.41	13.15	14.65	13.34	13.64	7.31	7.21	7.26	0.40	0.29	0.35
04.09.2018	32.40	32.50	32.45	12.13	14.09	15.10	13.26	13.65	7.09	7.15	7.12	0.20	0.22	0.21
08.09.2018	28.80	28.70	28.75	14.56	16.13	16.95	16.45	16.02	6.94	7.05	7.00	0.42	0.27	0.35
11.09.2018	25.10	25.30	25.20	14.04	15.10	14.02	15.02	14.55	7.04	7.09	7.07	0.30	0.32	0.31
13.09.2018	30.80	31.00	30.90	12.60	12.35	15.89	16.09	14.23	6.95	7.00	6.98	0.39	0.32	0.36
17.09.2018	29.90	30.00	29.95	15.77	14.83	16.70	14.70	15.50	7.06	7.14	7.10	0.31	0.30	0.31
21.09.2018	30.60	30.90	30.75	11.08	11.15	13.75	14.85	12.71	7.00	6.95	6.98	0.38	0.40	0.39
25.09.2018	26.50	26.50	26.50	14.70	11.50	13.70	14.58	13.62	7.06	7.06	7.06	0.25	0.39	0.32
28.09.2018	31.00	30.90	30.95	12.40	14.23	11.10	16.01	13.44	6.81	7.00	6.91	0.27	0.26	0.27
01.10.2018	31.50	30.90	31.20	15.50	14.65	14.20	14.67	14.76	7.02	7.00	7.01	0.25	0.30	0.28
05.10.2018	28.80	28.60	28.70	16.43	18.74	14.81	18.73	17.18	7.09	7.00	7.05	0.50	0.40	0.45
08.10.2018	30.30	30.10	30.20	16.60	14.00	13.75	14.40	14.69	6.92	6.89	6.91	0.43	0.30	0.37
12.10.2018	29.80	29.80	29.80	17.90	13.30	16.02	12.67	14.97	6.85	6.79	6.82	0.27	0.31	0.29
15.10.2018	28.90	28.90	28.90	15.45	11.85	13.80	13.70	13.70	6.79	6.82	6.81	0.26	0.47	0.37
19.10.2018	28.90	29.40	29.15	14.60	12.43	13.09	13.89	13.50	6.74	6.70	6.72	0.37	0.32	0.35

M1: 1st measurement, M2: 2nd measurement

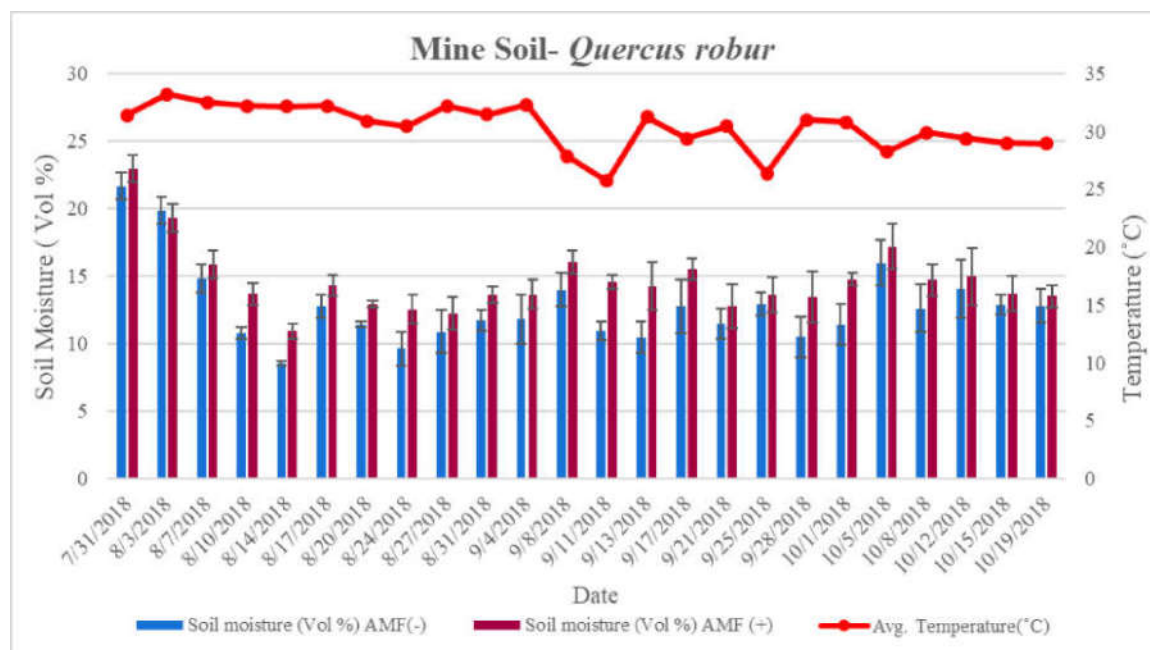


Figure 4.24. The comparison of soil moisture content between mine soil- AMF (+) *Quercus robur* pot and mine soil- AMF (-) *Quercus robur* pot

Measurements of salt content (AM) in mine soil- *Pinus nigra* AMF (-) pot demonstrated that the average salt content of the soil was 0.25g/L (± 0.075 g/L) (Table 4.9). Furthermore, the greenhouse temperature was usually 30 °C when soil moisture evaluation was done. Soil pH in mine soil- *Pinus nigra* AMF (-) pot was measured 6.72, which indicated soil pH was between 6.5 and 7.5, so it is a suitable value for plant growth due to the availability of nutrients.

Additionally, the same amount of water was given for all saplings throughout the greenhouse study. Soil moisture measurements demonstrated that moisture in mine soil- *Pinus nigra* AMF (-) pot was adjusted to lower than 15%, which was given Table 4.10 below, and it showed that saplings were under water stress.

Table 4.9. Measurements of soil moisture, external temperature, pH and salt content in mine soil- AMF (-) *Pinus nigra* pot

DATE	MINE SOIL- <i>Pinus nigra</i> AMF (-)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
24.07.2018	24.60	24.80	24.70	11.21	NA	10.01	NA	10.61	6.71	6.75	6.73	0.15	0.19	0.17
27.07.2018	30.30	30.50	30.40	17.50	NA	15.50	NA	16.50	7.01	7.05	7.03	0.42	0.40	0.41
31.07.2018	31.40	31.20	31.30	15.10	NA	14.20	NA	14.65	6.73	6.75	6.74	0.60	0.70	0.65
03.08.2018	32.10	32.30	32.20	14.00	NA	13.60	NA	13.80	6.86	6.92	6.89	0.70	0.70	0.7
07.08.2018	32.90	32.50	32.70	13.75	NA	13.05	NA	13.40	6.69	6.65	6.67	0.38	0.42	0.4
10.08.2018	32.10	32.40	32.25	15.70	NA	12.34	NA	14.02	6.46	6.43	6.45	0.03	0.27	0.15
14.08.2018	32.00	31.70	31.85	11.40	NA	6.67	NA	9.04	6.51	6.67	6.59	0.17	0.14	0.16
17.08.2018	32.70	32.40	32.55	10.70	NA	10.25	NA	10.48	6.54	6.61	6.58	0.05	0.49	0.27
20.08.2018	31.50	31.20	31.35	14.07	NA	11.11	NA	12.59	6.59	6.77	6.68	0.30	0.32	0.31
24.08.2018	31.80	31.90	31.85	5.40	15.36	6.95	14.21	10.48	6.85	6.85	6.85	0.27	0.13	0.20
27.08.2018	32.70	33.10	32.90	5.55	11.35	8.75	9.73	8.85	6.64	6.61	6.63	0.20	0.19	0.20
31.08.2018	31.10	31.50	31.30	9.45	7.71	7.09	7.21	7.87	6.67	6.58	6.63	0.41	0.29	0.35
04.09.2018	32.20	32.30	32.25	7.55	15.05	7.80	14.05	11.11	6.92	7.03	6.98	0.14	0.11	0.13
08.09.2018	28.80	29.40	29.10	9.35	18.90	11.76	10.30	12.58	6.89	6.64	6.77	0.27	0.13	0.20
11.09.2018	26.20	26.50	26.35	10.13	8.69	8.45	14.25	10.38	6.59	6.70	6.65	0.38	0.11	0.25
13.09.2018	31.30	31.70	31.50	8.84	8.23	9.36	7.66	8.52	6.60	6.75	6.68	0.21	0.28	0.25
17.09.2018	29.30	30.00	29.65	9.32	7.75	8.16	7.75	8.25	6.89	6.86	6.88	0.27	0.23	0.25
21.09.2018	30.40	30.30	30.35	14.60	8.76	10.50	12.40	11.57	6.70	6.75	6.73	0.12	0.11	0.12
25.09.2018	26.10	26.30	26.20	8.35	7.80	10.58	13.00	9.93	6.84	6.93	6.89	0.27	0.24	0.26
28.09.2018	31.40	31.50	31.45	9.40	8.35	7.55	6.85	8.04	6.73	6.84	6.79	0.31	0.24	0.28
01.10.2018	31.00	31.10	31.05	8.75	11.58	8.56	11.18	10.02	6.86	6.95	6.91	0.22	0.25	0.24
05.10.2018	28.40	28.70	28.55	10.71	12.34	12.05	9.85	11.24	6.95	7.00	6.98	0.49	0.30	0.40
08.10.2018	30.30	31.00	30.65	10.06	11.18	11.76	9.55	10.64	6.59	6.68	6.64	0.30	0.40	0.35
12.10.2018	29.50	30.00	29.75	9.30	9.36	9.40	13.44	10.38	6.70	6.71	6.71	0.25	0.30	0.28
15.10.2018	29.50	30.00	29.75	17.01	9.05	9.67	13.71	12.36	6.60	6.55	6.58	0.32	0.37	0.35
19.10.2018	29.00	28.50	28.75	10.22	15.45	8.40	15.51	12.40	6.63	6.75	6.69	0.28	0.46	0.37

M1: 1st measurement, M2: 2nd measurement

Measurements of soil moisture, temperature, pH, and salt content in mine soil- *Pinus nigra* AMF (+) pot was given in Table 4.10. The average mine soil salinity is 0.30g/L (± 0.075 g/L), which indicated mine soil salinity in the AMF (+) pot was higher than mine soil salinity in the AMF (-) pot (0.25 g/L). Also, the salinity in the mine soil was higher than in Muğla soil (0.10g/L). The temperature of the greenhouse was measured higher than 25 °C when evaluating soil moisture.

Soil pH in mine soil- *Pinus nigra* AMF (+) pot was measured 6.95, which was higher than mine soil pH in AMF (-) *Pinus nigra* pot, but it was a suitable value for plant growth due to near neutral. Measured soil moisture values demonstrated that the soil moisture in mine soil- *Quercus robur* AMF (+) pot was adjusted to lower than 20 % (Table 4.10).

Figure-4.25 demonstrates the comparison of soil moisture content between pots of mine soil- *Pinus nigra* AMF (+) and AMF (-). The results of the study indicated that soil moisture content of AMF (+) pot was superior to soil moisture in AMF (-) pot, although two pots were in the same conditions. According to the greenhouse study, mycorrhizal inoculation increased water uptake to the plant in mine soil- *Pinus nigra* AMF (+) pot under high temperature. The difference in soil moisture between mycorrhizal and non-mycorrhizal group can be seen clearly (Figure 4.25).

Table 4.10. Measurements of soil moisture, external temperature, pH and salt content in mine soil- AMF (+) *Pinus nigra* pot

DATE	MINE SOIL- <i>Pinus nigra</i> AMF (+)													
	Temp (°C)			Soil moisture (Vol %)					pH			AM (g/L)		
	1 st sapling	2 nd sapling	Avg.	1 st sapling		2 nd sapling		Avg.	1 st	2 nd	Avg.	1 st	2 nd	Avg.
				M1	M2	M1	M2							
24.07.2018	24.20	24.40	24.30	14.22	NA	13.20	NA	13.71	6.75	6.71	6.73	0.18	0.18	0.18
27.07.2018	30.40	30.60	30.50	19.90	NA	19.80	NA	19.85	7.26	7.20	7.23	0.70	0.76	0.73
31.07.2018	31.70	31.90	31.80	19.00	NA	17.10	NA	18.05	7.30	7.28	7.29	0.74	0.76	0.75
03.08.2018	32.70	32.70	32.70	19.10	NA	18.00	NA	18.55	7.40	7.30	7.35	0.72	0.78	0.75
07.08.2018	32.80	32.60	32.70	18.80	NA	18.10	NA	18.45	7.24	7.20	7.22	0.79	0.77	0.78
10.08.2018	31.50	31.60	31.55	19.60	NA	15.65	NA	17.63	6.75	6.69	6.72	0.14	0.45	0.30
14.08.2018	32.30	31.90	32.10	16.23	NA	10.13	NA	13.18	6.72	6.64	6.68	0.11	0.73	0.42
17.08.2018	30.10	30.10	30.10	10.48	NA	10.65	NA	10.57	7.07	7.06	7.07	0.07	0.08	0.08
20.08.2018	31.40	31.00	31.20	17.01	NA	18.50	NA	17.76	6.93	6.96	6.95	0.50	0.22	0.36
24.08.2018	30.40	30.50	30.45	11.53	15.07	7.75	14.57	12.23	6.83	6.96	6.90	0.28	0.11	0.20
27.08.2018	32.50	32.80	32.65	12.10	14.25	9.20	15.47	12.76	6.81	6.97	6.89	0.29	0.11	0.20
31.08.2018	32.20	32.30	32.25	13.02	11.43	10.67	13.85	12.24	6.97	7.15	7.06	0.64	0.32	0.48
04.09.2018	32.90	32.70	32.80	13.40	15.56	12.07	14.70	13.93	7.27	7.21	7.24	0.12	0.13	0.13
08.09.2018	28.50	28.80	28.65	19.11	13.23	13.23	17.90	15.87	6.97	7.03	7.00	0.14	0.12	0.13
11.09.2018	25.40	25.50	25.45	9.30	14.35	17.54	12.15	13.34	7.09	7.06	7.08	0.37	0.18	0.28
13.09.2018	31.40	31.00	31.20	9.12	7.39	17.21	9.91	10.91	6.86	6.95	6.91	0.28	0.20	0.24
17.09.2018	27.80	28.75	28.28	10.45	8.71	10.60	16.67	11.61	6.89	7.00	6.95	0.26	0.48	0.37
21.09.2018	30.50	30.50	30.50	10.90	9.02	10.20	19.15	12.32	6.75	6.95	6.85	0.22	0.50	0.36
25.09.2018	26.30	26.70	26.50	8.39	15.50	11.20	17.85	13.24	6.95	7.06	7.01	0.26	0.43	0.35
28.09.2018	31.10	31.00	31.05	10.40	10.35	9.50	15.51	11.44	6.92	7.03	6.98	0.39	0.39	0.39
01.10.2018	31.30	31.40	31.35	14.88	13.04	9.36	14.90	13.05	7.00	7.00	7.00	0.27	0.39	0.33
05.10.2018	29.90	29.20	29.55	17.40	13.34	19.40	11.05	15.30	7.04	7.09	7.07	0.73	0.30	0.52
08.10.2018	30.20	30.40	30.30	15.74	11.85	11.10	20.75	14.86	6.92	7.00	6.96	0.26	0.41	0.34
12.10.2018	29.90	29.80	29.85	18.26	11.03	19.85	12.75	15.47	6.90	7.01	6.96	0.27	0.30	0.29
15.10.2018	29.10	28.70	28.90	15.51	10.30	20.21	13.60	14.91	6.82	6.98	6.90	0.49	0.20	0.35
19.10.2018	28.80	28.80	28.80	15.65	10.50	16.54	12.40	13.77	6.88	6.82	6.85	0.40	0.20	0.30

M1: 1st measurement, M2: 2nd measurement

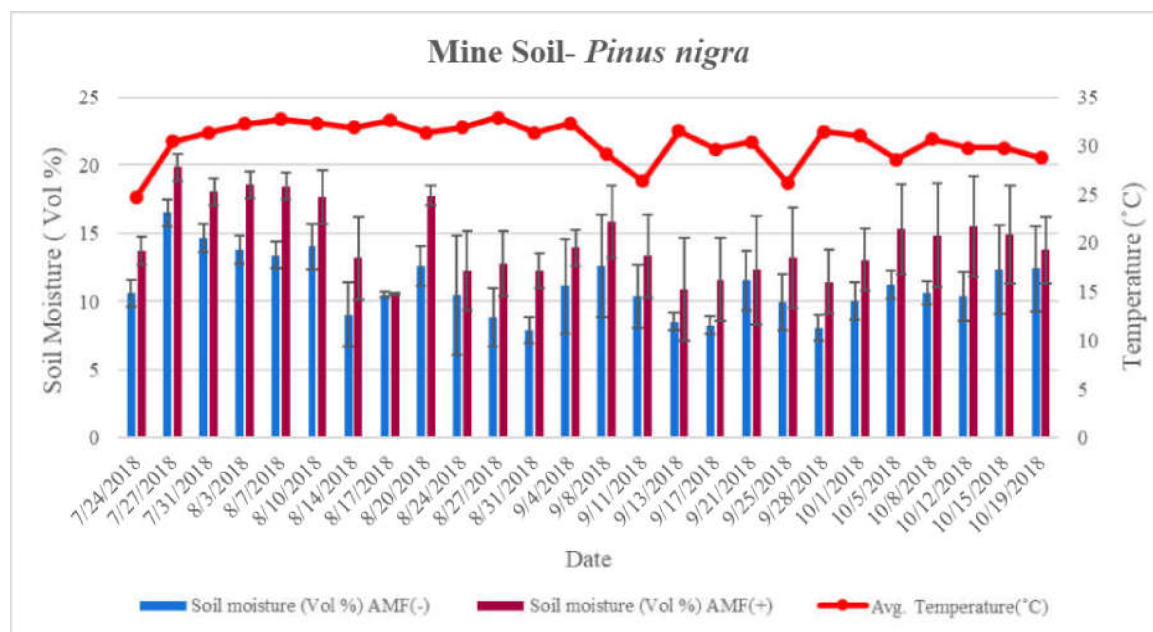


Figure 4.25. The comparison of soil moisture content between mine soil- AMF (+) *Pinus nigra* pot and mine soil- AMF (-) *Pinus nigra* pot

According to the result of the t-test, there was a statistically significant difference ($0,007 < 0.05$) between soil moisture rates of AMF (+) and control group (AMF (-)) pot cultures. Two-way ANOVA test also indicated that AMF ($P=0.003$), soil types ($P=0.000$), and AMF-soil ($P=0.001$) had a statistically significant effect on soil moisture. On the other hand, the effects of AMF-tree species ($P=0.876$) and AMF-soil-tree species ($P=0.055$) on soil moisture were not significant in two-way ANOVA test using SPSS v.22 (IBM Corp, 2013). The greenhouse study showed that AMF used pots both in Muğla soil and mine soil are more successful when water uptake as well as reducing drought stress in their host plants. Thus, soil microbial contributions would be increased in the forest areas to decrease drought stress during dry summer and it would be used as a precaution to prevent or to slow down forest fires in arid regions. Besides, soil mycorrhizal inoculum can be used in the mining region for reforestation.

4.2.4. Soil Electrical Conductivity Measurements

Soil salinity is the most significant factor which influences plant growth and soil fertility since salt stress leads to decrease uptake of essential nutrients and water use efficiency (Miransari, 2017). Electrical conductivity (EC) measurements of water-saturated soil paste extract are used to decide soil salinity (Table 4.11) (Artiola et al., 2019).

Table 4.11. Soil salinity rankings

Parameter (mScm ⁻¹)	Nonsaline	Slightly Saline	Moderately Saline	Saline
EC	<4	4 - 8	8 - 16	>16

In the study, the electrical conductivity (EC) of Muğla soil were evaluated by utilizing COMBI 5000 (Table 4.12, Table 4.13). The results of EC values of soils collected from the Muğla forest area indicated that soil used in the study was not salty. Also, low values of AM measurements of soils supported it.

Table 4.12. EC measurements of Muğla soil- *Quercus petraea* pots

MUĞLA SOIL- <i>Quercus petraea</i>				
	<i>Quercus petraea</i> AMF (+) (close tree)	<i>Quercus petraea</i> AMF (+) (middle of pot)	<i>Quercus petraea</i> AMF (-). (close tree)	<i>Quercus petraea</i> AMF (-). (middle of pot)
Soil (g)	4.0346	4.0558	4.0052	4.0413
Distilled Water (mL)	40	40	40	40
EC (mS / cm)	0.1280	0.1950	0.070	0.1010
Avg. EC (mS /cm)	0.1615		0.0855	

Table 4.13. EC measurements of Muğla soil - *Pinus nigra* pots

MUĞLA SOIL- <i>Pinus nigra</i>				
	<i>Pinus nigra</i> AMF (+) (close tree)	<i>Pinus nigra</i> AMF (+) (middle of pot)	<i>Pinus nigra</i> AMF (-). (close tree)	<i>Pinus nigra</i> AMF (-). (middle of pot)
Soil (g)	4.0107	4.0025	4.048	4.0661
Distilled Water (mL)	40	40	40	40
EC (mS / cm)	0.090	0.111	0.155	0.350
Avg. EC (mS /cm)	0.1005		0.2525	

The measurements of electrical conductivity of Muğla soil collected from *Quercus petraea* and *Pinus nigra* pots at the end of the study demonstrated that it was non-saline (< 4 mS/cm) (Figure 4.26). The salt content of the samples was quantified using COMBI 5000 twice a week throughout the greenhouse study, and also average values of AM measurements of soils promoted it. Furthermore,

the EC value in *Pinus nigra* AMF (-) pot was measured higher than in AMF (+) pot. On the other hand, the EC value in *Quercus petraea* AMF (+) pot was evaluated higher than in AMF (-) pot (Figure 4.26). This situation may be related to AMF inoculation, which may affect salt intake in saplings differently or it could directly be related to tree species.

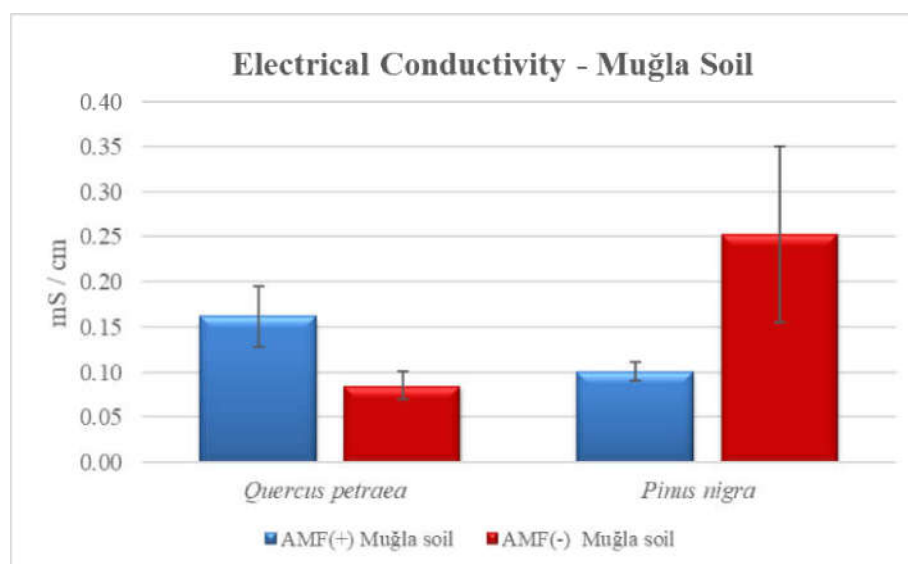


Figure 4.26. EC results of Muğla soil – *Quercus petraea* and *Pinus nigra* pots

The electrical conductivity (EC) of mine soil were evaluated via utilizing COMBI 5000 (Table 4.14, 4.15). The results of EC values of soils collected from the mining area indicated that soil utilized in the study was non-saline. Low values of AM measurements of soils also supported it.

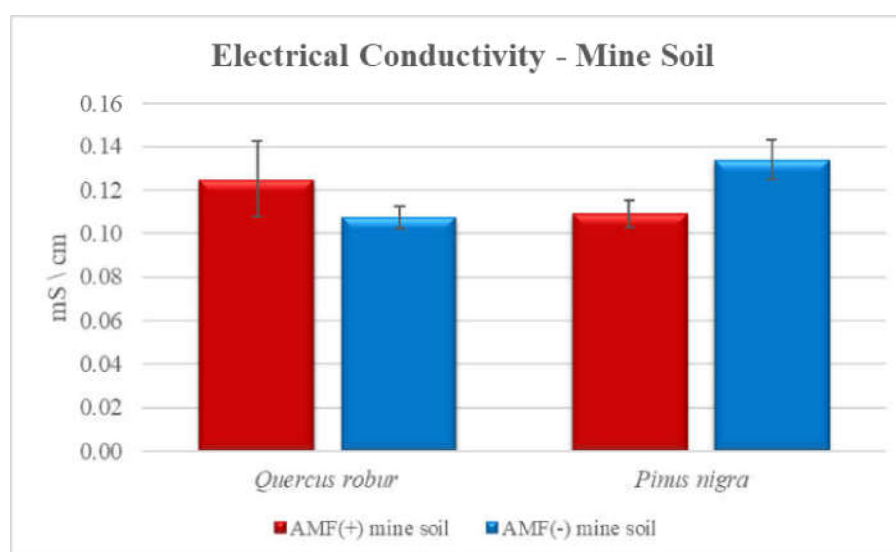
Table 4.14. EC measurements of mine soil- *Quercus robur* pots

	MINE SOIL-<i>Quercus robur</i>			
	<i>Quercus robur</i> AMF (+) (close tree)	<i>Quercus robur</i> AMF (+) (middle of pot)	<i>Quercus robur</i> Control G. (close tree)	<i>Quercus robur</i> Control G. (middle of pot)
Soil (g)	4.0170	4.0055	4.0189	4.0190
Distilled Water (mL)	40	40	40	40
EC (mS/cm)	0.120	0.130	0.090	0.125
Avg. EC (mS/cm)	0.125		0.108	

Table 4.15. EC measurements of mine soil - *Pinus nigra* pots

	MINE SOIL-<i>Pinus nigra</i>			
	<i>Pinus nigra</i> AMF (+) (close tree)	<i>Pinus nigra</i> AMF (+) (middle of pot)	<i>Pinus nigra</i> Control G. (close tree)	<i>Pinus nigra</i> Control G. (middle of pot)
Soil (g)	4.0775	4.0350	4.0266	4.0182
Distilled Water (mL)	40	40	40	40
EC (mS/cm)	0.118	0.100	0.128	0.140
Avg. EC (mS/cm)	0.109		0.134	

The evaluations of EC of mine soil collected from *Quercus petraea* and *Pinus nigra* pots at the end of the study showed that salinity was low (< 4 mS/cm) (Figure 4.27). The AM values of the samples were measured utilizing COMBI 5000 twice a week throughout the greenhouse study, and average values of AM measurements of soils promoted that soil was non-saline. Furthermore, the EC value in *Pinus nigra* AMF (-) pot was measured higher than in AMF (+) pot. On the other hand, the EC value in *Quercus petraea* AMF (+) pot was evaluated higher than in AMF (-) pot (Figure 4.27). This situation may be related to AMF inoculation, which may affect salt intake in saplings differently or it could directly be related to tree species.

Figure 4.27. EC results of *Quercus robur* and *Pinus nigra* pots

The results showed that EC values of both soils collected from the mining region and soil collected from the Muğla forest area were lower and it promotes that the soil was not salty. Also, low values of AM measurements of the soil supported it.

4.2.5. Determination of Mycorrhization

The effects of AMF were studied along with their mycorrhization (colonization) rates in sapling roots. AMF colonization rates were measured microscopically by using the root staining method with trypan blue. The highest colonization rate was in mine soil- *Pinus nigra* AMF (+) symbioses by *G. mosseae* species with 40.2% root colonization, which were given in Table 4.16. Moreover, mycorrhization rates indicated that root colonization rates in AMF (+) groups were higher than in control groups.

Table 4.16. Average mycorrhization rates of pot cultures

Sample name	Average Mycorrhization (%)
Muğla Soil - <i>Quercus petraea</i> AMF (-)	8.80
Muğla Soil - <i>Quercus petraea</i> AMF (+)	21.40
Muğla Soil - <i>Pinus nigra</i> AMF (-)	7.50
Muğla Soil - <i>Pinus nigra</i> AMF (+)	18.70
Mine Soil - <i>Quercus robur</i> AMF (-)	17.70
Mine Soil - <i>Quercus robur</i> AMF (+)	20.90
Mine Soil - <i>Pinus nigra</i> AMF (-)	11.10
Mine Soil - <i>Pinus nigra</i> AMF (+)	40.20

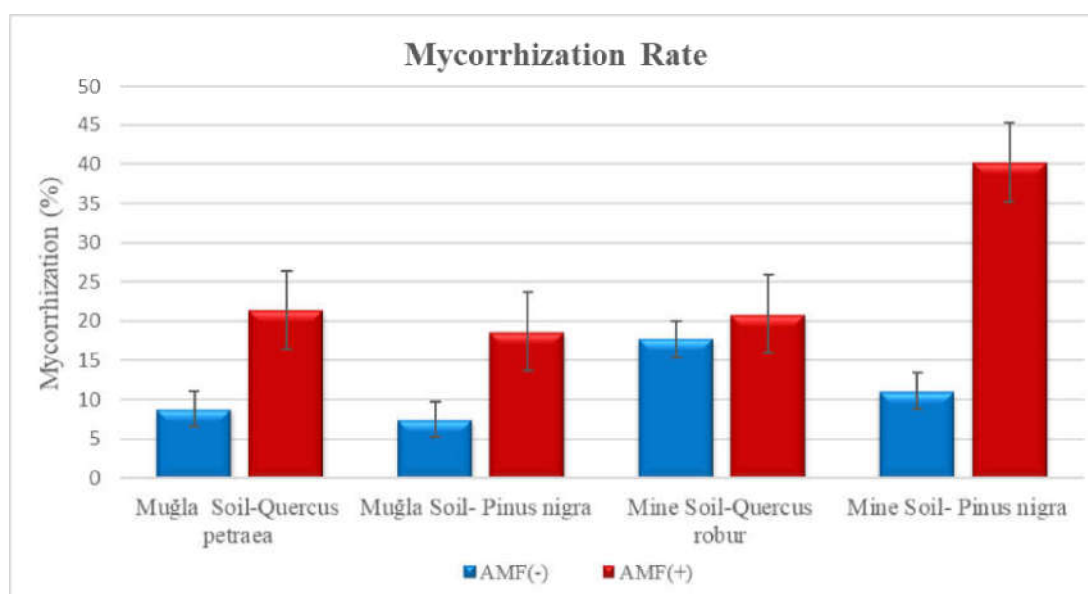


Figure 4.28. Mycorrhization rate of pot cultures

Arbuscular mycorrhizal fungi spread via the roots and interaction between AMF and sapling roots in Muğla soil groups were given in Figure 4.29 and Figure 4.30. Results indicated that water

stress and high temperature increased AMF colonization. Root colonization of infected plants ranged from 8.8% to 21.4% in Muğla Soil - *Quercus petraea* pot cultures and increased from 7.5% to 18.7% in Muğla Soil - *Pinus nigra* pot cultures (Table 4.24)

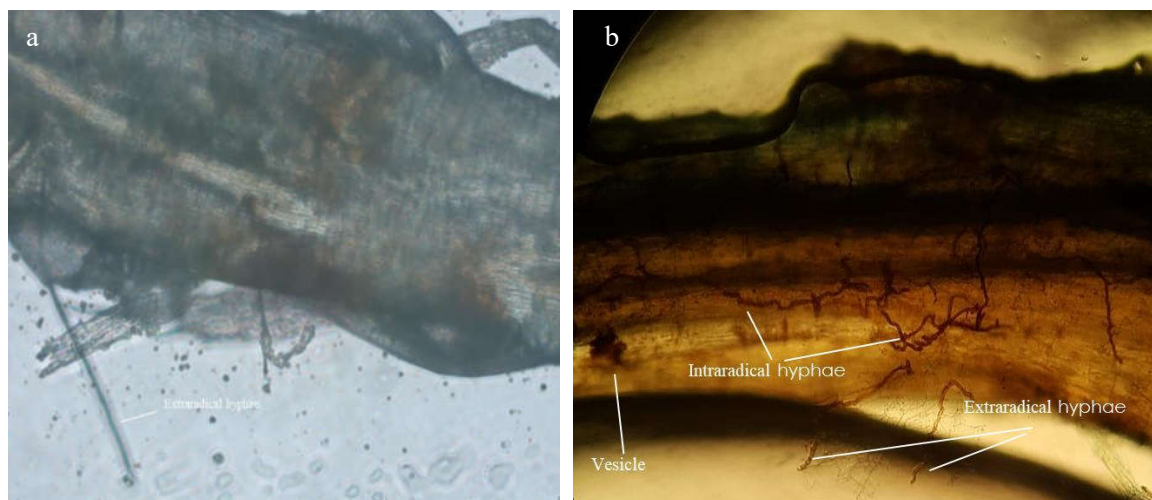


Figure 4.29. Muğla *Quercus petraea* AMF (-) (a) and Muğla *Quercus petraea* AMF (+) (b)

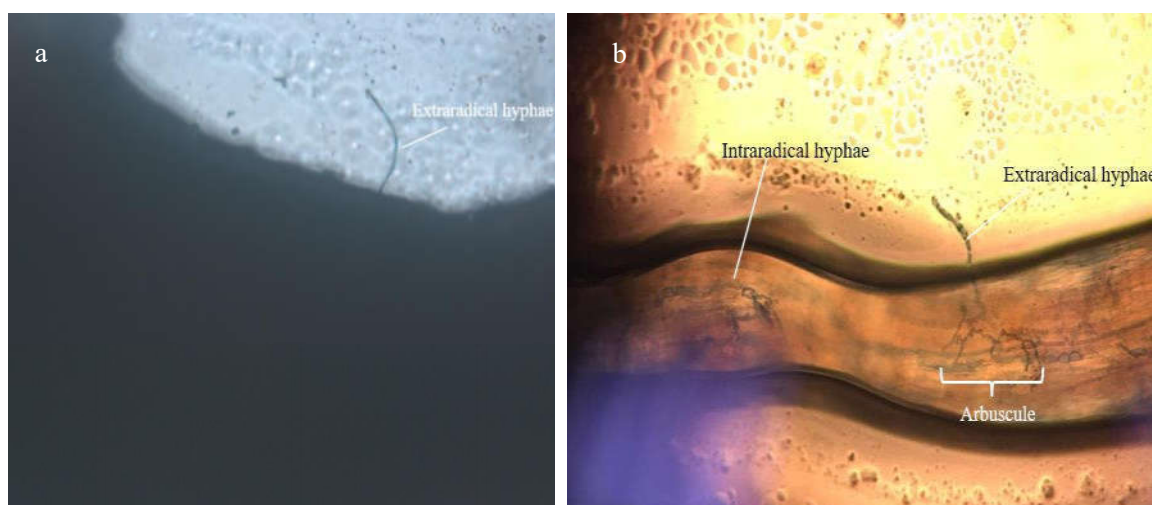


Figure 4.30. Muğla *Pinus nigra* AMF (-) (a) and Muğla *Pinus nigra* AMF (-) (b)

AMF spread by the roots and interaction between AMF and sapling roots in mine soil groups were given in Figure 4.31 and Figure 4.32. Results showed that water deficiency and the high temperature raised AMF colonization. Root colonization of infected plants ranged from 17.7% to 20.9% in mine Soil - *Quercus petraea* pot cultures and increased from 11.1% to 40.2% in mine Soil - *Pinus nigra* pot cultures (Table 4.16)

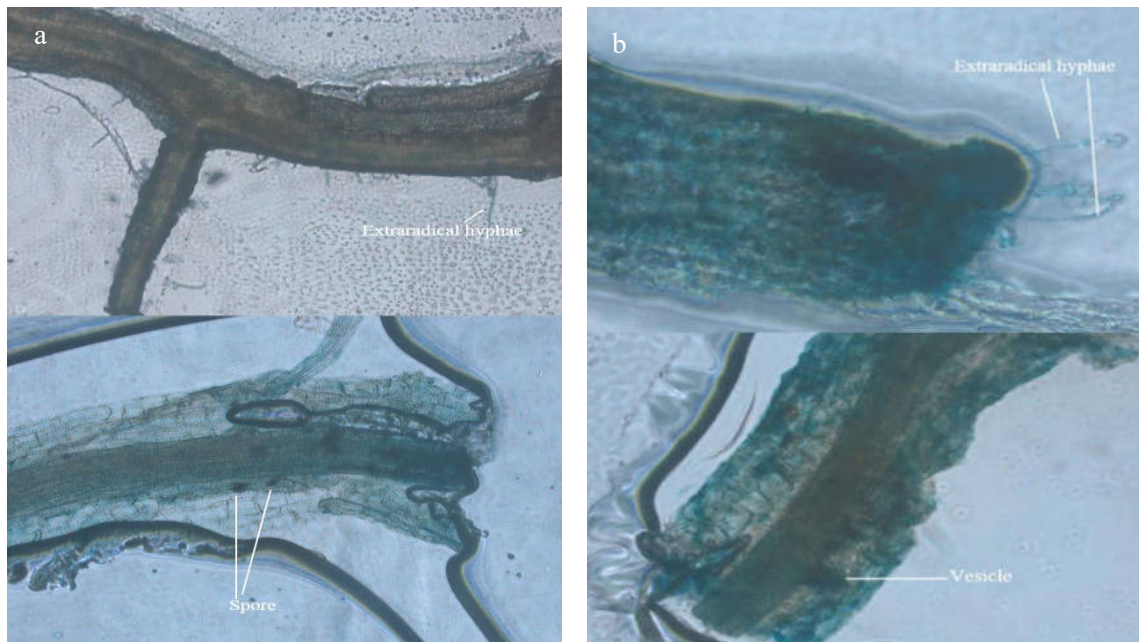


Figure 4.31. Mine *Quercus robur* AMF (-) (a) and Mine *Quercus robur* AMF (+) (b)

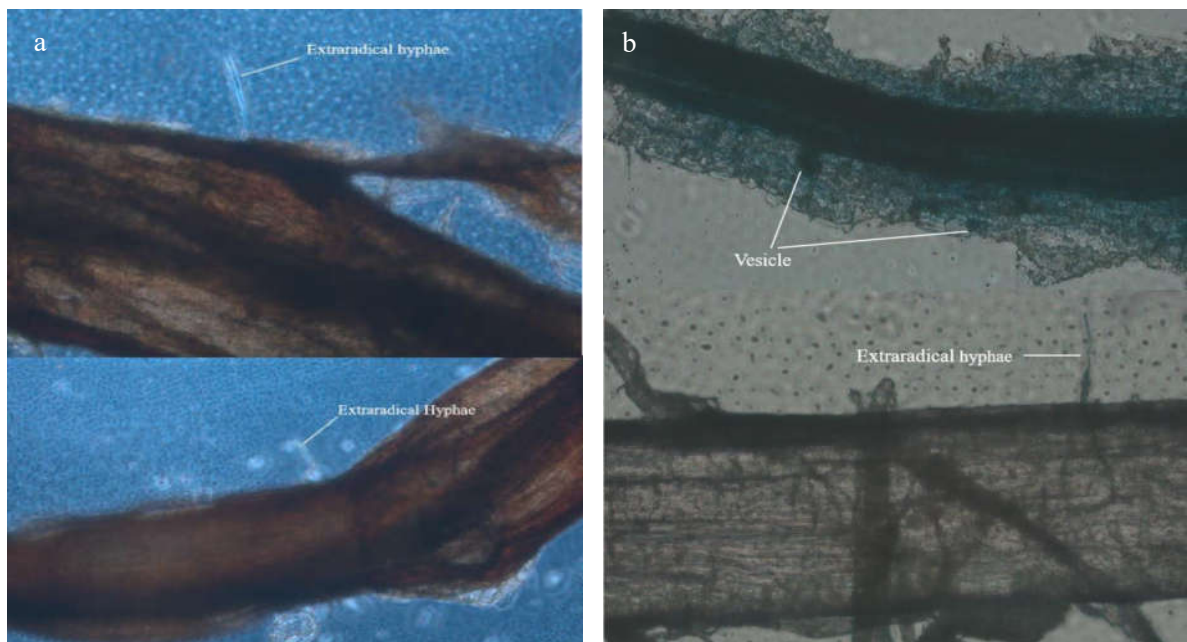


Figure 4.32. Mine *Pinus nigra* AMF (-) (a) and Mine *Pinus nigra* AMF (+) (b)

Arbuscular mycorrhizal trees are predominant in a seasonal, warm tropical forest, and occur with ectomycorrhizal trees in temperate biomes where seasonally warm and wet climates increase decomposition. Research about the sensitivity of global patterns of tree symbiosis to climate change indicates the great number of ectomycorrhizal trees will decrease (as plentiful as 10%) by using the

projected climates for 2070 according to the RCP8.5 concentration scenario and the largest declines in ectomycorrhizal affluence will take place along the boreal–temperate ecotone, where small rises in climatic decomposition coefficients lead to abrupt transitions to arbuscular mycorrhizal forests (Steidinger et al., 2019). Therefore, arbuscular mycorrhizal fungi will start to be crucial in the forests where arbuscular mycorrhizal and ectomycorrhizal trees coexist due to decreasing several ectomycorrhiza

In the study, the results of root colonization rates in control groups both in Muğla soil and in mine soil demonstrated that AMF is found naturally in soils. On the other hand, AMF inoculation increased mycorrhization rates in plant roots and promoted plant growth under water stress and high temperature. The greenhouse study demonstrates that AMF can be used not only for reforestation in the mining region but also for increasing drought tolerance in the forest area. Furthermore, a great number of ectomycorrhiza will decrease in the future due to extreme temperatures. AMF could reduce the potential adverse effects of loss of ectomycorrhizal trees in the forest biome in the future.

4.2.6. Measurement of Glomalin Related Soil Protein (GRSP)

Total Glomalin Related Soil Protein (TGRSP) was assessed by Bradford Assay. Before the analysis of TGRSP, the spectrophotometer was calibrated with the standard protein solution (P0834-10x1mL; Sigma; 2mg/mL; Lot SLBS3852). Absorbance values of the standard solution and constructed calibration curve are demonstrated in Table 4.17 and Figure 4.33 respectively.

Table 4.17. Absorbance values of shown the standard protein solution at 595 nm.

Concentration of protein standard (mg/mL)	Absorbance (595nm)
0.0	0.000
0.2	0.119
0.4	0.222
0.6	0.402
0.8	0.535
1.0	0.666

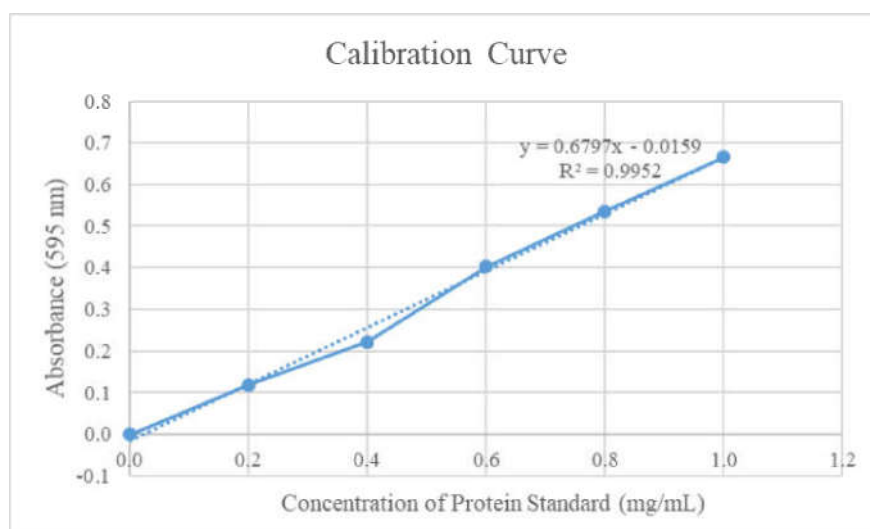


Figure 4.33. Calibration curve of the standard protein solution

In Figure 4.33, the R^2 value is 0.9952, which demonstrates a nearly perfect linear curve was obtained. In this way, protein concentrations of the extracts were calculated correctly according to this calibration curve. Furthermore, the total amount of protein per sample (mg/g) and amount of protein per kg sample were calculated by basing on the volume of citric acid per sample in which the extracts were present and the amount of taken sample respectively. The calculated values are shown in Table 4.18.

Table 4.18. Calculated protein concentration values of samples

Sample name	Absorbance at 595nm	Concentration of protein in sample in mg/mL	Total amount of protein in 8mL sample in mg/g	Amount of mg protein per kg sample
Muğla <i>Quercus petraea</i> AMF (-)	0.423	0.6457	5.1658	5165.8
Muğla <i>Quercus petraea</i> AMF (+)	0.467	0.7105	5.6837	5683.7
Muğla <i>Pinus nigra</i> AMF (-)	0.214	0.3382	2.7059	2705.9
Muğla <i>Pinus nigra</i> (1) AMF (+)	0.221	0.3485	2.7883	2788.3
Muğla <i>Pinus nigra</i> (2) AMF (+)	0.219	0.3456	2.7647	2764.7
Mine <i>Quercus robur</i> AMF (-)	0.21	0.3324	2.6588	2658.8
Mine <i>Quercus robur</i> (1) AMF (+)	0.356	0.5472	4.3772	4377.2
Mine <i>Quercus robur</i> (2) AMF (+)	0.353	0.5427	4.3419	4341.9
Mine <i>Pinus nigra</i> AMF (-)	0.161	0.2603	2.0821	2082.1
Mine <i>Pinus nigra</i> (1) AMF (+)	0.291	0.4515	3.6122	3612.2
Mine <i>Pinus nigra</i> (2) AMF (+)	0.245	0.3838	3.0708	3070.8

Protein content in soil and root is demonstrated in Figure 4.34 below.

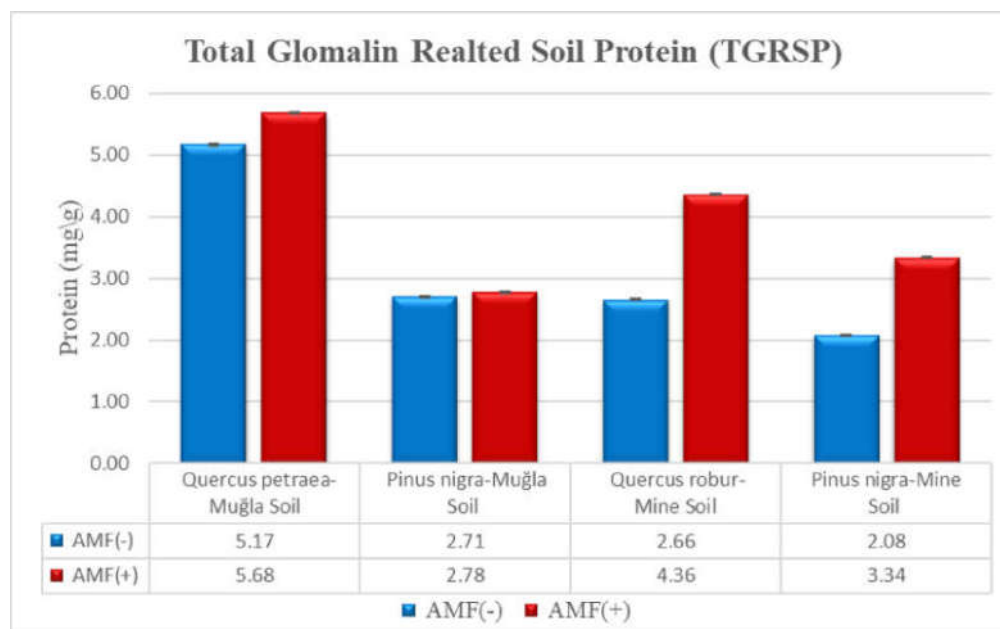


Figure 4.34. Average protein content in soil and root

The results of TGRSP extracted from soil and root show that the amount of glomalin in AMF (+) pot cultures were higher than in control groups (Table 4.18). Two-way ANOVA test indicated that AMF-soil ($P=0.004$) and AMF-species ($P=0.002$) had a statistically significant effect on the amount of glomalin. On the other hand, AMF alone had no significant effect on TGRSP ($P> 0.05$) (SPSS v.22 IBM Corp, 2013). Production rates of glomalin were estimated from 3 months of greenhouse study. Short-period greenhouse experiments do not last long enough for glomalin pools to turn over substantially (Shing et al., 2013). Therefore, the difference in TGRSP between some of the control and AMF (+) groups was less than expected such as Muğla soil-*Pinus nigra* AMF (+) and AMF (-) group.

Moreover, soil texture affects the yields of glomalin (Rillig and Steinberg, 2002) and the result of the study provided that an increase in the amount of TGRSP in mine soil AMF (+) pot cultures are higher than Muğla soil groups. Thus, the difference between the control and AMF (+) group is seen clearly (Figure 4.33). It could be related to soil collected from coal mine includes a higher amount of carbon (C) than Muğla soil (Table 4.19) since a significant amount of C promotes glomalin productivity (Treseder and Turner 2007).

Furthermore, this short-term greenhouse study demonstrated that AMF can be used not only for reforestation in the mining region but also for increasing soil moisture in the forest area and also decrease the atmospheric concentration of CO₂ and N₂O since higher amounts of glomalin promote the aggregate stability of soils which modify the soil's ability to mobilize nutrients, water content, as well as root penetration in soil and soil erosion potential. Also, glomalin can contribute to decreasing in the release of both N₂O and CO₂ into the atmosphere (Shing et al., 2013).

4.2.7. Elemental Analysis of Soil

AMF is in symbiotic association with host plants and has a positive effect on soil carbon and nitrogen accumulation (Zhang et al., 2017). Table 4.19 shows that nitrogen concentrations of soil samples from AMF (+) pot cultures were higher than the results of control groups except for the Muğla soil-*Pinus nigra* group.

On the other hand, the results of elemental analysis in the study indicated that the total carbon concentrations of soils collected from AMF (+) pots were lower than total carbon concentrations of control groups. A decrease in soil carbon and a rise in TGRSP in the short-term study under higher temperatures could be related to the rising in biomass and activity of microorganisms in rhizosphere soil (Huang et al., 2016; Jia et al., 2018). Also, the growth of saplings in AMF (+) pots was higher than the growth of saplings in control groups.

Table 4.19. Average values of elemental analysis of soil samples

Sample	Amount of sample(mg)	Nitrogen		Carbon	
		Weight (mg)	Weight (%)	Weight (mg)	Weight (%)
Muğla soil	1.5845	0.0030	0.180	0.0785	4.935
Muğla soil - <i>Pinus nigra</i> AMF (-)	1.3505	0.0020	0.135	0.0600	4.425
Muğla soil - <i>Pinus nigra</i> AMF (+)	1.6375	0.0020	0.095	0.0465	2.855
Muğla soil - <i>Quercus petraea</i> AMF (-)	1.5670	0.0040	0.250	0.0885	5.665
Muğla soil - <i>Quercus petraea</i> AMF (+)	1.5300	0.0040	0.265	0.0855	5.655
Mine soil - <i>Pinus nigra</i> AMF (-)	1.6395	0.0020	0.120	0.0770	4.695
Mine soil - <i>Pinus nigra</i> AMF (+)	1.4145	0.0020	0.125	0.0595	4.195
Mine soil - <i>Quercus robur</i> AMF (-)	1.4860	0.0020	0.125	0.0960	6.430
Mine soil - <i>Quercus robur</i> AMF (+)	1.4130	0.0020	0.130	0.0880	6.235

5. CONCLUSION

The impact of climate change on forest fires in the Mediterranean basin has been increasing in recent years. The basis of this issue lies in the fact that summers start to be hot and dry due to climate change in the regions where we observe the Mediterranean climate. This situation has already caused an increase in the number of forest fires. Many studies related to wildland fires also indicate that the number of forest fires will significantly rise in the future. Wildfires mean not only the burning of trees but also the damage to living organisms. For this reason, it is crucial to forecast the regions where forest fires could occur by using forest fire indices. In this way, necessary precautions could be taken in these regions.

The Canadian Fire Weather Index (FWI) is one of the most preferred indices to estimate wildland fire disasters since the FWI system associates weather information with fuel moisture and fire risk indices. Additionally, this system is adapted to different forest types. FWI has also currently been used as the official index for fire danger predictions issued by the European Forest Fire Information System. Thus, in this study, FWI was used to present a reliable forecast of extreme fire seasons in the MENA region. It could be used for forest fire management.

The results of FWI re-forecasts by MPI-ESM-MR and HadGEM2-ES models show that the output of MPI-ESM-MR provides more accurate fire risk prediction than the result of HadGEM2-ES based on the historical hindcast of observational data (ERA-Interim). Thus, the MPI-ESM-MR model could be more suitable for making a skillful FWI forecast according to the preliminary assessment.

The outputs of FWI forecasts for the future period 2070-2099 with respect to the reference period 1971-2000 based on the IPCC's RCP4.5 and RCP8.5 (worst case) scenarios were presented. It is obvious that the future projections indicate a significant rise in the risk of forest fires along the region for the last 30 years of this century.

FWI-MPI-ESM-MR forecast output under the low-concentration scenario RCP4.5 in the southern and western parts of Turkey, Greece, the southern part of Italy, France, the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the southern part of Iberian Peninsula (Spain, Portugal) indicate high-level forest fire risk according to EFFIS FWI classification. Additionally, the result of FWI-HadGEM2-ES projection under the low-concentration scenario RCP4.5 in these regions and the Balkans show very high-level fire risk.

FWI-MPI-ESM-MR forecast output under the high-concentration scenario RCP8.5 in the southern and western parts of Turkey, Greece, the southern part of Italy, France, the northwestern part of Morocco, the northern part of Algeria and Tunisia, and the southern part of Iberian Peninsula (Spain, Portugal) indicate very high-level forest fire risk concerning EFFIS FWI classification. Moreover, the result of FWI- HadGEM2-ES projection under the worst-case scenario RCP8.5 in these regions and the Balkans demonstrate extreme-level fire risk.

In the greenhouse study, plant-microbe (AMF) interaction was investigated to increase soil moisture under water stress and high temperature throughout three months since arbuscular mycorrhizal fungi (AMF) is one of the microbial symbionts significantly affect the functioning of forest ecosystems by improving the ability of water uptake from soil and reducing drought stress in their host plants. In this experiment, soil moisture rate was measured by using soil collected from the Muğla forest area and a mine tailing area in Kütahya, *Pinus nigra* (Turkish pine) sapling, *Quercus* (oak) sapling and mycorrhiza (AMF). The consequence of the study shows that AMF enhances plant growth, symbiotic relations, and drought tolerance in the host plants.

Besides, according to the results of soil moisture measurements, AMF (+) pot cultures in Muğla soil and mine soil are more successful when water uptake in their host plants. There is a significant difference between the soil moisture measurements of AMF (+) pot cultures and AMF (-) pot cultures. Thus, AMF could be inoculated forest areas to increase or protect soil moisture.

Furthermore, the ANOVA test indicated that AMF-soil and AMF-species together had a statistically significant effect on total glomalin related soil protein (TGRSP) which supports the aggregate stability of soils by modifying the soil's ability to mobilize nutrients, water content, and root penetration in soil erosion potential.

In the greenhouse study, sapling in Muğla soil- *Quercus petraea* (oak) AMF (-) pot was affected by powdery mildew disease which is a fungal disease. However, saplings in Muğla soil- *Quercus petraea* (oak) AMF (+) pot was not infected by powdery mildew disease even though saplings were closer to infected plants since the soil in this pot included both iron (Fe) and AMF. Fe has an important role in the formation of glomalin and iron in the organic matter could contribute to the thermal stability and antimicrobial properties of glomalin.

Consequently, the forest vegetation in the Mediterranean basin is also relatively rich with the Mediterranean climate. Fire activity is strongly affected by four factors: fuels, climate–weather,

ignition agents, and people. Climate change causes to increase drought weather conditions and extreme temperatures in the Mediterranean region. Thus, the FWI analysis presents important information for estimating the burned area and the number of fires under future climate scenarios. Moreover, soil microbial contributions might be increased in a forest area to decrease drought stress as a precaution for forest fires during dry summer.

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APPENDIX: FWI CODE

```
#This code developed by Burcu Calda and Kamil Çöllü at Institute of Environmental Sciences
#and at Center for Climate Change and Policy Studies at Bogazici University for this main purpose.
#You can implement this code for other datasets like MPI and HadGEM2.
#Please ignore warnings unless they are errors.

setwd("path_your_directory")

#loading needed libraries
library(chron)
library(RColorBrewer)
library(lattice)
library(ncdf4)
library(transformeR)
library(downscaleR)
library(visualizeR)
library(fireDanger)
library(easyVerification)
library(R.matlab)
library(rworldmap)
library(maps)

#coordinates of our domain lon = longitude, lat = latitude.
lon = seq(from = -12, to = 53, length.out = 148)
lat = seq(from = 30, to = 50, length.out = 40)

load("Tmobs.rdata") #loading example data from
http://meteo.unican.es/work/fireDanger/Climate\_Services\_2017.html
#loading created dates for this time range according to example data
```

```

dates2 <- readMat("str6.mat")
dates3 = dates2[["str6"]]
dates= drop(dates3)
#loading and creating temperature ERAINTERIM data from our simulation output for time period
tas <- readMat("eratas0510.mat")
attr(tas, "header") <- NULL
tas=tas[["tas0510"]]
attr(tas, "dimensions") <- c("time", "lat", "lon")
Tm.obs2 = Tm.obs
Tm.obs2[["Data"]]=tas
Tm.obs2[["xyCoords"]][["x"]]=lon
Tm.obs2[["xyCoords"]][["y"]]=lat
Tm.obs2[["Dates"]][["start"]]=dates
Tm.obs2[["Dates"]][["end"]]=dates

#loading and creating relative humidity ERAINTERIM data from our simulation for time period
hurs <- readMat("erarh0510.mat")
attr(hurs, "header") <- NULL
hurs=hurs[["rh0510"]]
attr(hurs, "dimensions") <- c("time", "lat", "lon")
H.obs2 = Tm.obs
H.obs2[["Data"]]=hurs
H.obs2[["xyCoords"]][["x"]]=lon
H.obs2[["xyCoords"]][["y"]]=lat
H.obs2[["Dates"]][["start"]]=dates
H.obs2[["Dates"]][["end"]]=dates
H.obs2[["Variable"]][["varName"]]='hursmin'

#loading and creating wind ERAINTERIM data from our simulation for time period
wss <- readMat("erawind0510.mat")

```

```

attr(wss, "header") <- NULL
wss=wss[["wind0510"]]
attr(wss, "dimensions") <- c("time", "lat", "lon")
W.obs2 = Tm.obs
W.obs2[["Variable"]][["varName"]]='wss'
W.obs2[["Data"]]=wss
W.obs2[["xyCoords"]][["x"]]=lon
W.obs2[["xyCoords"]][["y"]]=lat
W.obs2[["Dates"]][["start"]]=dates
W.obs2[["Dates"]][["end"]]=dates
W.obs2[["Variable"]][["varName"]]='wss'

#loading and creating precipitation ERAINTERIM data from our simulation for time period
tp <- readMat("erapr0510.mat")
attr(tp, "header") <- NULL
tp=tp[["pr0510"]]
attr(tp, "dimensions") <- c("time", "lat", "lon")
r.obs2 = Tm.obs
r.obs2[["Data"]]=tp
r.obs2[["xyCoords"]][["x"]]=lon
r.obs2[["xyCoords"]][["y"]]=lat
r.obs2[["Dates"]][["start"]]=dates
r.obs2[["Dates"]][["end"]]=dates
r.obs2[["Variable"]][["varName"]]='tp'

#creating multigrid
multigrid_obs2 <- makeMultiGrid(Tm.obs2, H.obs2, r.obs2, W.obs2)
obs2 <- fwiGrid(multigrid = multigrid_obs2)

#setting colors

```

```
fwi.colors <- colorRampPalette(c(rev(brewer.pal(9, "YlGnBu")[3:5]),
                                brewer.pal(9, "YlOrRd")[3:9]))

#getting cliobs
cliobs=climatology(obs2)
ee2=cliobs[["Data"]]
ee2=drop(ee2)
ee3=t(ee2)

#Adding world map
newmap <- getMap(resolution = "low")
plot(newmap, xlim = c(-12, 53), ylim = c(30, 50), asp = 1)

#contour the image with world map
filled.contour(ee3, x=lon, y=lat, levels = c(0,5,10,15,20,25,30,35,40,45,50,55,60,65,70,75,80), plot.title =
title(main = "ERA-Interim FWI (1980-2012)", color.palette = fwi.colors, plot.axes = {map(add = T)}))
```