Soil microbial diversity in agriculture

Responses to land-use and extreme weather events

Kozjek, Katja

2022

Document Version:
Other version

Soil microbial diversity in agriculture

Responses to land-use and extreme weather events

KATJA KOZJEK
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY

Soil microbial diversity in agriculture

Agricultural land-use intensification and increased occurrence and intensity of extreme weather events like droughts are two of the main threats responsible for soil biodiversity declines and further changes in their ecosystem functions. Microbial diversity is an essential key for the understanding of ecosystem functioning, however the diversity of functions performed by soil microorganisms and how they are linked to ecosystem functions like carbon cycling remain largely unexplored. This thesis provides a deeper understanding of how the diversity of soil microorganisms is influenced by agricultural land-use intensification and drought. These findings highlight that modified agricultural land-use practices have the potential to reduce the negative effects of drought on soil microorganisms, soil functions and further soil ecosystem processes.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

Department of Biology
Faculty of Science

ISBN 978-91-8039-132-0

Soil microbial diversity in agriculture

Soil microbial diversity in agriculture

Responses to land-use and extreme weather events

Katja Kozjek

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Science, Lund University, Sweden.
To be defended at Blue Hall, Ecology building, Sölvegatan 37, Lund, Sweden on the 11th February 2022 at 10:00.

Faculty opponent
Professor George Kowalchuk
Utrecht University, Institute of Environmental Biology, Department of Biology

Organization
LUND UNIVERSITY
Department of Biology
Sölvegatan 37, 22362 Lund, Sweden
Author: Katja Kozjek

Document name: Doctoral dissertation

Date of issue: 11th February 2022

Sponsoring organization

Title and subtitle: Soil microbial diversity in agriculture: Responses to land-use and extreme weather events

Abstract

Land-use intensification of agricultural soils and increased occurrence and intensity of extreme weather events like drought periods are two of the main threats responsible for soil biodiversity declines. These changes in soil biodiversity can alter ecosystem functions performed especially by soil microbial communities that could further contribute to those threats. Microbial diversity is an essential key for the understanding of ecosystem functioning, however the diversity of functions performed by soil microorganisms and how they are linked to ecosystem functions like carbon cycling remain largely unexplored.

The aim of this thesis was to understand how the taxonomic and functional diversity of soil microorganisms in agriculture are influenced by agricultural land-use intensification and extreme weather events, specifically short-term drought. Thus, a combination of field experiments across Europe and glasshouse experiments along with different molecular methods, specifically high-throughput sequencing-based omics approaches was used.

Different land-use types (grassland and agricultural soils) affected soil microbial communities, particularly their response in relation to soil organic matter degradation. It was found that crop management practices, i.e., crop residue incorporation promoted gene expression in these soils, particularly in agricultural soils. These findings support the notion that careful land-use practices have the potential to mitigate losses of soil organic carbon in traditionally carbon depleted soils and can thereafter promote the functioning of soil microorganisms. Further, interactive effects of long-term agricultural management and short-term drought on the communities of plant-associated arbuscular mycorrhizal fungi (AMF) were studied. Organic and conventional long-term farming systems influenced the taxonomic composition of AMF, while the effects on their diversity were negligible. No effect of short-term drought on the diversity and composition of AMF was found. To further explore how short-term drought influence the functional diversity of soil microorganisms in agricultural soils, particularly on the gene level, functional genetic diversity was assessed. By studying the diversity of extracellular enzymes related to soil organic matter degradation, it was found that functional and taxonomic gene composition significantly differed between European agricultural fields (Sweden, Germany, and Spain). However, the effect of short-term drought was only observed in Germany. These results indicate that soil microorganisms are differently adjusted to short-term drought, either due to (a) regional adaptations of microorganisms to already dry environments or (b) differences in soil physicochemical properties like soil organic carbon content, as it has the potential to buffer drought effects. Finally, the short-term drought also affected the response of soil microbial communities in these soils, especially in their gene expression towards degrading soil organic matter.

Altogether, these findings show that soil microorganisms respond differently to agricultural land-use intensification and extreme weather events such as drought. Careful land-use practices like the incorporation of crop residues, specific farming systems and increased levels of soil organic carbon have the potential to mitigate the negative effects of drought on soil health and soil microorganisms. Moreover, these findings demonstrate the importance of studying microbial responses to drought at different diversity levels, with the necessity to link taxonomic and functional diversity to soil ecosystem functions.

Key words: agriculture, drought, soil microbial communities, taxonomic diversity, functional diversity

Classification system and/or index terms (if any)

Supplementary bibliographical information — **Language:** English

ISSN and key title — **ISBN:**
978-91-8039-131-3 (pdf)
978-91-8039-132-0 (print)

Recipient's notes — **Number of pages** 187 — Price

Security classification

Signature Katja Kozjek Date 2022-01-03

Soil microbial diversity in agriculture

Responses to land-use and extreme weather events

Katja Kozjek

LUND
UNIVERSITY

Cover by Ema Ločniškar

Faculty of Science
Department of Biology

ISBN 978-91-8039-131-3 (pdf)
978-91-8039-132-0 (print)

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

Verjel sem v pot in dosegel cilj, ki ga sploh nisem imel.
~Nejc Zaplotnik

Table of Contents

List of Papers

I. **Kozjek, K.***, Manoharan, L.*, Urich, T., Ahrén, D., Hedlund, K. *Straw addition promotes microbial activity in agricultural soils comparable to grasslands.* Manuscript.

II. **Kozjek, K.**, Kundel, D., Kushwaha, S.K., Olsson, P.A., Ahrén, D., Fliessbach, A., Birkhofer, K., Hedlund, K. 2021. *Long-term agricultural management impacts arbuscular mycorrhizal fungi more than short-term experimental drought*. Applied Soil Ecology 168, 104140.

III. **Kozjek, K.**, Manoharan, L., Ahrén, D., Hedlund, K. *Microbial functional genes influenced by short-term experimental drought across European agricultural soils.* Manuscript (submitted).

IV. **Kozjek, K.**, Manoharan, L., Jørgensen, H.B., Ahrén, D., Hedlund, K. *Microbial responses to short-term experimental drought in agricultural soils*. Manuscript.

* These authors contributed equally to this work.

Author contributions

I. LM, DA, KH conceptualized and designed the study. LM performed the laboratory work and bioinformatic analyses. **KK** contributed to bioinformatics and was responsible for the statistical analyses. TU contributed with inputs to the laboratory work. **KK** outlined and wrote the manuscript with inputs from all authors.

II. **KK**, PAO, DA and KH designed the AMF study. **KK** conducted the molecular laboratory work, the bioinformatical and statistical analyses. DK conducted fieldwork, provided plant- and soil-related data and contributed to the statistical analyses. SKK contributed to the bioinformatical analysis. The design of the experiment was a part of a larger European project where KB was responsible for the rainout shelter design and overall experiment. **KK** outlined and wrote the manuscript with inputs from all authors.

III. **KK** and KH designed and carried out the study that was a part of a larger European wide experiment, with the input from DA. **KK** performed the laboratory work, bioinformatic and statistical analyses. LM contributed with inputs to the bioinformatic analyses. **KK** wrote the paper with contributions from all authors.

IV. **KK** and KH designed the study. **KK** performed the experiment and HBJ the soil PLFA analyses. **KK** conducted the laboratory work, bioinformatic and statistical analyses. LM contributed to the bioinformatic analyses. DA and KH provided overall guidance throughout the work. **KK** wrote the manuscript with inputs from all authors.

KK – Katja Kozjek

LM – Lokeshwaran Manoharan

DA – Dag Ahrén

KH – Katarina Hedlund

PAO – Pål Axel Olsson

DK – Dominika Kundel

KB – Klaus Birkhofer

TU – Tim Urich

HBJ – Helene Bracht Jørgensen

SKK – Sandeep K. Kushwaha

List of abbreviations

AA auxiliary activities
AMF arbuscular mycorrhizal fungi
ASV amplicon sequence variant
BIODYN biodynamic
CAZymes Carbohydrate-Active enZYmes
CBM/CBMF carbohydrate-binding module
CCS circular consensus sequence
cDNA complementary deoxyribonucleic acid
CE carbohydrate esterase
CONMIN conventional
DOK trial (bio**d**ynamic, bio**o**rganic and conventional [**k**onventionell])
DNA deoxyribonucleic acid
GH glycoside hydrolase
GT glycosyltransferase
ITS internal transcribed spacer
LSU large subunit
mRNA messenger ribonucleic acid
PCR polymerase chain reaction
PL polysaccharide lyase
RNA ribonucleic acid
rRNA ribosomal ribonucleic acid
SOC soil organic carbon
SOM soil organic matter
SMRT single-molecule real-time sequencing
SSU small subunit
WHC water holding capacity

Abstract

Land-use intensification of agricultural soils and increased occurrence and intensity of extreme weather events like drought periods are two of the main threats responsible for soil biodiversity declines. These changes in soil biodiversity can alter ecosystem functions performed especially by soil microbial communities that could further contribute to those threats. Microbial diversity is an essential key for the understanding of ecosystem functioning, however the diversity of functions performed by soil microorganisms and how they are linked to ecosystem functions like carbon cycling remain largely unexplored.

The aim of this thesis was to understand how the taxonomic and functional diversity of soil microorganisms in agriculture are influenced by agricultural land-use intensification and extreme weather events, specifically short-term drought. Thus, a combination of field experiments across Europe and glasshouse experiments along with different molecular methods, specifically high-throughput sequencing-based omics approaches was used.

Different land-use types (grassland and agricultural soils) affected soil microbial communities, particularly their response in relation to soil organic matter degradation. It was found that crop management practices, i.e., crop residue incorporation promoted gene expression in these soils, particularly in agricultural soils. These findings support the notion that careful land-use practices have the potential to mitigate losses of soil organic carbon in traditionally carbon depleted soils and can thereafter promote the functioning of soil microorganisms. Further, interactive effects of long-term agricultural management and short-term drought on the communities of plant-associated arbuscular mycorrhizal fungi (AMF) were studied. Organic and conventional long-term farming systems influenced the taxonomic composition of AMF, while the effects on their diversity were negligible. No effect of short-term drought on the diversity and composition of AMF was found. To further explore how short-term drought influence the functional diversity of soil microorganisms in agricultural soils, particularly on the gene level, functional genetic diversity was assessed. By studying the diversity of extracellular enzymes related to soil organic matter degradation, it was found that functional and taxonomic gene composition significantly differed between European agricultural fields (Sweden, Germany, and Spain). However, the effect of short-term drought was only observed in Germany. These results indicate that soil microorganisms are differently adjusted to short-term drought, either due to (a) regional adaptations of

microorganisms to already dry environments or (b) differences in soil physicochemical properties like soil organic carbon content, as it has the potential to buffer drought effects. Finally, the short-term drought also affected the response of soil microbial communities in these soils, especially in their gene expression towards degrading soil organic matter.

Altogether, these findings show that soil microorganisms respond differently to agricultural land-use intensification and extreme weather events such as drought. Careful land-use practices like the incorporation of crop residues, specific farming systems and increased levels of soil organic carbon have the potential to mitigate the negative effects of drought on soil health and soil microorganisms. Moreover, these findings demonstrate the importance of studying microbial responses to drought at different diversity levels, with the necessity to link taxonomic and functional diversity to soil ecosystem functions.

Popular science summary

Soils are material on which we build roads, construct buildings, walk and grow plants. However, soils are much more than this. Soils harbour an extraordinary portion of the biodiversity on our planet, including tiny living things called microorganisms. They are too small to be seen by the naked eye and are visible only through a microscope. Despite their size, they are extremely important for our health, industries and environment. A single handful of soil is home to more microorganisms than there are stars in the universe. These microorganisms form communities, different microorganisms together, and are involved in different ecosystem functions such as nutrient cycling through degradation of soil organic matter that comes from plant residues, animal manure and compost. During the degradation process, soil microorganisms degrade these organic materials and provide nutrients like nitrogen, phosphorus, and sulphur to plants. On the other hand, these microorganisms obtain organic carbon that gets stored in soils. High levels of stored organic carbon can improve soil structure, reduce erosion, promote soil biodiversity, and have the potential to mitigate the effects of climate change.

Due to the growing human population and the need to produce enough food, agricultural practices worldwide are being intensified. Farmers need to increase crop production, either by application of chemical fertilizers, pesticides, or modification of existing farming practices. In combination with global climate change, specifically increased occurrence and frequency of extreme weather events such as drought, this can cause several environmental problems and can reduce soil biodiversity. Losses of soil biodiversity can further lead to a reduction of soil functioning and soil services carried out by soil microorganisms, such as climate regulation, nutrient cycling, and food production. High microbial diversity is important for supporting ecosystem functions and services, therefore, the need to protect soil biodiversity and functions performed by soil microorganisms is essential. To find a way towards more sustainable agriculture in the long run, it is thus critical to understand how anthropogenic factors influence the diversity of soil microorganisms and how this is linked to ecosystem processes.

The diversity of soil microorganisms determining the functioning of the soil ecosystem is enormous, and thereafter challenging to study. The majority of soil microorganisms cannot be cultivated in the laboratory, however researchers have developed new methods that allow studying the full extent of soil microbial diversity directly from soils. Collection and analysis of genetic material (DNA or RNA) from

soils and targeting of soil microorganisms and their specific functions like those related to the degradation of soil organic material allow us to establish relationships between soil microbial diversity, their functions and soil ecosystem processes.

In this thesis, I studied how the intensification of agriculture and extreme weather events, especially droughts, affected the diversity of soil microorganisms and their functions.

First, the effects of different land-use types (grassland and agricultural soils) on the active microorganisms were studied. The results of this study showed that the overall gene expression related to organic matter degradation differed between the two land-uses. Moreover, the addition of organic materials, i.e., crop residues promoted the expression of genes in traditionally carbon depleted soils like agricultural soils. With time, the gene expression of these microorganisms seemed similar to that of the carbon rich soils, i.e., grasslands. This suggests that the addition of organic compounds can increase the activity of soil microorganisms in soils with lower amounts of organic carbon content and indicate the potential way towards healthier soils and increased productivity.

Second, I wanted to understand how different farming systems and drought simultaneously affect arbuscular mycorrhizal fungi. These fungi are of crucial importance in agriculture, as they colonize the roots of plants, such as cereals, including wheat and barley. They transfer water and nutrients from the soil to the plants and in return they receive carbon. Additionally, this association could enhance plant tolerance to environmental stress like drought. I showed that the drought did not influence the diversity and community composition of these fungal communities. While different farming systems altered their community composition but not the diversity. These findings suggest that these soil microorganisms are capable of coping with drought, however more attention should be paid to how current agricultural practices could affect them. As agricultural practices can potentially enhance the resistance of these fungal communities to drought and possibly maintain high crop production levels under drought periods.

Finally, I wanted to understand how drought affects the diversity of soil microorganisms in agricultural soils across Europe, specifically on their functional gene level. By studying the genetic information of different soil microorganisms that carry out specific biological processes, we can obtain information of their genetic potential and their response to changes in environmental factors of only active microorganisms. It was found that functional genetic diversity differed between European agricultural fields, furthermore drought effects had varying effects on soil microorganisms. Some microorganisms better tolerated drought, which was probably due to regional adaptations of these microorganisms to already dry environments or because of increased soil organic carbon content that can reduce the effects of drought. Additionally, I also showed that drought affected active soil microbial communities in these soils.

In summary, this thesis provides a deeper understanding of how the diversity of soil microorganisms is influenced by agricultural land-use intensification and drought. Agricultural land-use intensification and physicochemical properties are important factors determining the diversity of soil microorganisms in agriculture. These findings highlight that modified agricultural land-use practices have the potential to reduce the negative effects of drought on soil microorganisms, soil functions and further soil ecosystem processes.

Introduction

Agricultural intensification and extreme weather events caused by the changing climate are strongly affecting soil ecosystem and soil biodiversity (Cavicchioli et al., 2019; de Graaff et al., 2019; Geisen et al., 2019). Soils harbour a large portion of biological diversity on Earth, including microorganisms (e.g., bacteria), micro- (e.g., nematodes, tardigrades), meso- (e.g., collembolans, mites) and macrofauna (e.g., ants, earthworms) (Orgiazzi et al., 2016). These soil organisms are crucial for multiple ecosystem functions and services, including nutrient cycling, carbon sequestration, climate regulation, and food provision (Wall et al., 2012; Bardgett and van der Putten, 2014). Despite the importance of soil biodiversity for ecosystem functioning, we still face major challenges in understanding the ecology of soil microorganisms, their diversity and relationship with soil ecosystem. Particularly, the question remains on how the agricultural intensification and extreme weather events influence links between specific soil microbial taxa and soil processes.

Soil ecosystem

Soils as a microbial habitat

Soils represent a complex and highly dynamic ecosystem on our planet, serving as the main reservoir for distinct microorganisms, including bacteria, fungi, archaea, protozoa, and viruses (Fierer, 2017; Jansson and Hofmockel, 2020). Although these tiny organisms are largely invisible to the naked eye, they drive crucial ecosystem functions such as nutrient cycling, and the degradation of soil organic matter (SOM). They also form symbiotic relationships with plant roots and thereby play an important role in the maintenance of soil fertility and plant productivity (van der Heijden et al., 2008; Crowther et al., 2019). Given the importance of soil microorganisms for soils, less is known on how the disturbances (natural and anthropogenic) affect their diversity and consequently the ecosystem processes they mediate.

Carbon cycling and soil organic matter degradation

SOM represents one of the largest reservoirs of organic carbon and nitrogen on our planet and its turnover plays a crucial role in global element cycling (Batjes, 1996; Schmidt et al., 2011). Soil organic carbon (SOC) is the main source of carbon nutrients required for microbial life and plant productivity. SOC can regulate physicochemical soil properties, can improve soil stability, water holding capacity, provide more plant nutrients and increase soil biodiversity (Lal, 2016; Minasny et al., 2017). However, as the pool of SOC is sensitive to global climate and land-use changes this can lead to limited carbon sequestration capacity (Zomer et al., 2017). Amounts of SOM and carbon influence soil fertility, health, and functioning, therefore a better understanding of biological mechanisms and players involved in carbon cycling is crucial. Abiotic factors, like moisture and temperature are considered as primary determinants of SOM degradation and carbon cycling, but soil microorganisms are greatly responsible for it (Nielsen et al., 2011). Soil microorganisms, particularly fungi and bacteria, produce enzymes that then catalyse specific reactions that are part of SOM degradation and are responsible to gain nutrients and energy for soil microorganisms that they use for enzyme production (Sinsabaugh and Follstad Shah, 2012; Burns et al., 2013). Thus, the production and activity of enzymes are directly linked to ecosystem functioning. Among all these enzymes, there has been particular interest in the role of extracellular enzymes, broadly produced by soil microorganisms. They have been proposed as proximate agents of processes related to SOM as their activity is crucial for ecosystem functioning, particularly degradation and mineralization of SOM (Sinsabaugh et al., 2008). Most soil microorganisms have extracellular enzymes to degrade labile carbon sources such as simple carbohydrates, but others have enzymes to degrade complex carbon substrates such as hemicellulose and lignin (Eichorst and Kuske, 2012). The phenotypic and genotypic microbial diversity help microorganisms to degrade different substrates (Ettema and Wardle, 2002; Crawford et al., 2012). The production and efficiency of extracellular enzymes in soils mainly depend on the substrate and energy availability for the microorganisms and soil physicochemical properties (Wallenstein and Burns, 2011). However, how biological, chemical and physical properties of soils and consequences of climate change and agricultural disturbances affect soil microorganisms and their extracellular enzyme production, is still poorly understood.

Threats to soil biodiversity

Agricultural intensification and extreme weather events as the consequence of the ongoing global climate change present major threats to soil ecosystems, leading to losses of SOC, soil biodiversity and consequently to reduced agricultural production (de Graaff et al., 2019; Geisen et al., 2019). With a need to produce enough food to

feed the world, a greater level of understanding of how interactive effects of agricultural intensification and extreme weather events affect soil microbial diversity, and the ecosystem functions they mediate, is required. This knowledge will contribute to the understanding of how agriculture could be adapted to mitigate the adverse effects of climate extremes, preserve soil biodiversity in the long-term and ensure sustainable agricultural production.

Agricultural land-use intensification

Soils are sensitive to changes in land-use and agricultural management practices. Indeed, these practices then modify soil properties and lead to soil biodiversity losses (Foley et al., 2005; Tsiafouli et al., 2015). Grasslands and agricultural soils are two common land-use types in agriculture, that among others act as important reservoirs of carbon and soil biodiversity. Land-use has been identified as one of the main factors affecting soil microorganisms, their diversity, and functions (Oehl et al., 2010; Manoharan et al., 2017a; Madegwa and Uchida, 2021). Land-use either has a direct effect on soil microorganisms or indirectly influences soil physicochemical properties and plant diversity (Lauber et al., 2008; Thomson et al., 2015; Delgado-Baquerizo et al., 2016; Manoharan et al., 2017a; Kardol and De Long, 2018). Compared to grasslands, agricultural soils are traditionally depleted in their SOC content and are also more prone to further losses of SOC, due to agricultural practices they are commonly associated with (Lal, 2013).

Direct effects of land-use on soil microbial communities are the result of chemical disturbances like crop protection strategies, e.g., application of pesticides, and fertilizers, and mechanical disturbances like tillage (Yang et al., 2021). A large body of research suggests that for example application of mineral (NPK) fertilizers have negative effects on soil microbial communities, including the diversity of arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) (Smith and Read, 2010; Lin et al., 2012; de Graaff et al., 2019). However, on the other hand, mineral fertilization increases crop yields (Yousaf et al., 2017). Mechanical soil disturbances such as tillage typically alter the structure and functioning of AMF communities, resulting in the breakdown of hyphal networks, reduced abundance of spores, lower taxonomic diversity, and reduced biomass (Helgason et al., 1998; Jansa et al., 2002; Schnoor et al., 2011).

To prevent arable soils from becoming poorly productive in the long run, and to mitigate the drought effects, different aspects of land-use and agricultural practices should be considered. Consequently, different agricultural implications with a goal to improve nutrient cycling, promote SOC storage, and ensure the right amounts of nutrients to soils, have been suggested (Bolinder et al., 2020; Lessmann et al., 2021).

Crop residue incorporation

Practices such as crop residue incorporation have been commonly used to promote carbon sequestration, particularly in agricultural soils (Powlson et al., 2008; Bolinder et al., 2020). Crop residue such as straw from cereal crops represent an important carbon and nitrogen source in agriculture, thus its application results in multiple benefits for arable soils (Liu et al., 2014). It aims to increase SOM, with resulting positive impacts on soil structure, reduced evaporation, increased soil fertility, higher crop yields, and improved other physical and biological properties (Rengel, 2007; Lal et al., 2011). This should potentially create favourable conditions for microbial growth, as well as sufficient carbon, nitrogen supplies and energy which could improve microbial diversity and activity (Jin et al., 2020).

Farming systems

To reduce the negative effects of agricultural land-use intensification on the soil microbial communities, alternative farming systems such as organic farming have been proposed (McLaughlin and Mineau, 1995). Organic farming aims at obtaining high-quality crop yields while maintaining soil biodiversity in the long-term (Birkhofer et al., 2016; Rundlöf et al., 2016). However, the beneficial effects of organic farming on biodiversity often come at the cost of lower crop yields (Seufert et al., 2012). Numerous studies have shown positive effects of organic farming on soil fertility (Mäder et al., 2002), SOC content (Gattinger et al., 2012; García-Palacios et al., 2018) as well as microbial biomass and diversity (Esperschutz et al., 2007; Hartmann et al., 2015; Lori et al., 2017). Potential advantages of organic farming compared to conventional have also been associated with enhanced AMF diversity (Verbruggen et al., 2010; Manoharan et al., 2017b). However, the question remains if organic farming, aiming at buffering extreme drought events through promoting SOC levels, also can enhance the drought tolerance of AMF communities.

Extreme weather events

Climate change factors that include increased occurrence of extreme weather events such as droughts, floods, higher levels of CO_2 and temperature extremes are unavoidable phenomena that are and will affect soil ecosystems and soil biodiversity globally (Cavicchioli et al., 2019; Jansson and Hofmockel, 2020). In many European regions, the frequency and severity of extreme drought events are expected, particularly during the growing season (Spinoni et al., 2018; Vogel et al., 2019; Masson-Delmotte, 2021). Drought-induced changes can lead to shifts in soil microbial communities, their taxonomic and functional diversity and consequently lead to alterations of ecosystem functions driven by soil microorganisms (Hueso et al., 2012; Deveautour et al., 2018; Ochoa-Hueso et al., 2018; Schimel, 2018). The drought effects on soil microbial communities largely depend on the duration,

frequency and intensity of drought events or the historical precipitation regimes (Hoover and Rogers, 2016; Meisner et al., 2018; Preece et al., 2019).

To survive harsh conditions in dry soils, microbial communities have developed different strategies. Generally, soil microbial communities in arid and semiarid environments are better adapted and known to be more drought tolerant (Acosta-Martínez et al., 2014; Maestre et al., 2015). Some of these important survival strategies include the ability to adapt to changes in water potential and low resource conditions, thicker cell walls and high resistance to desiccation (Sharma and Gobi, 2016; Barberán et al., 2017; Schimel, 2018). Among all soil microorganisms, fungal communities and fungal based food-webs are generally thought to be more drought tolerant (de Vries et al., 2012; de Vries et al., 2018). Fungi such as AMF that have symbiotic relationships with plant roots in agricultural systems, including cereals (Schüβler et al., 2001) form filamentous structures called mycorrhizal hyphae. These extended hyphal networks can exceed several meters in diameter and can also enter water-filled soil pores inaccessible to root hairs and enhance the drought tolerance of their symbiotic partner, plant roots (Marulanda et al., 2003; Ruiz-Lozano, 2003). These characteristics allow them to survive better in dry environments (Allen, 2007; Manzoni et al., 2012).

The adverse impacts of drought on the microbial life in soils can also be mitigated through improved land-use, particularly through increased SOC levels. SOC has the ability to increase the capacity to hold water and nutrients, enhance soil biological and physical properties such as aggregate stability, improve soil structure, minimize degradation risk and soil erosion (Lal, 2013; Iizumi and Wagai, 2019). Higher SOC levels promote soil microbial diversity and activity and can buffer the negative effects of drought events on crop yields (Birkhofer et al., 2012; Droste et al., 2020). Even though promoting SOC levels can increase the resistance of soil microorganisms to drought and lead to higher crop yields, arable soils are under considerable risk due to agricultural intensification that reduces SOC content and limits carbon sequestration (Lal et al., 2011).

Taxonomic and functional diversity

An essential attribute of soil microorganisms is their diversity, as it enhances soil ecosystem functions and their tolerance to disturbances such as land-use or fertilizer application (Griffiths and Philippot, 2013; Bender et al., 2016). Every gram of soil contains thousands of microbial taxa and kilometres of fungal hyphae, however, they are not equally abundant (Fierer, 2017). A high degree of taxonomic diversity and high relative abundance of microbial taxa make soils one of the most complex and unexplored ecosystems, hence far less is known about the functional capabilities of microorganisms found in soils (Torsvik and Øvreås, 2002; Maron et al., 2011).

Microbial ecologists have only recently started recognizing the importance of soil microbial functional diversity, i.e., diversity of all functions carried out by microorganisms (Escalas et al., 2019). Precise functional characterization of soil microbial communities could fill an essential but still largely missing link between soil microbial diversity and microbially-mediated processes important for soil functioning (Fig. 1). At present, the functional diversity of soil microorganisms is mostly derived from taxonomy-based studies, however, this information can be misleading. As such, taxonomically distinct groups like bacteria and fungi competing for the same nutrient sources could share very similar functions or closely related microbial taxa could possess different functions (Philippot et al., 2010; Martiny et al., 2013; Bahram et al., 2018). To understand microbial functional capabilities at the highest level of resolution, and how they are linked to ecosystem functions such as carbon cycling, studying genes within the soil environment was suggested (Manoharan et al., 2017a). These genes, the so-called functional genes, are coding for key enzymes in biogeochemical processes, such as the degradation of organic matter in soils (Prosser, 2002).

Approaches to study soil microbial diversity

One of the main challenges in soil microbial ecology is to understand the taxonomic, functional, and ecological characteristics of soil microbial communities more precisely. Until a few years ago, it has been difficult to obtain precise functional information from microbial communities and to consequently predict microbial functional responses to disturbances such as agricultural intensification or extreme weather events. However, the continued development of high-throughput molecular tools (high-throughput sequencing-based omics approaches) allows us to study functional diversity at a high resolution and link it with ecosystem functions.

Microbial taxonomic diversity

One of the most widely and affordable methods for the characterization of soil microbial communities is based on the amplification and sequencing of specific highly conserved taxonomic marker genes (Fig. 1). The most commonly used marker genes are 16S for bacteria and archaea (Langille et al., 2013), ITS for fungi (Schoch et al., 2012) and 18S for microbial eukaryotes (Popovic and Parkinson, 2018). The ITS region is often used to describe fungal communities, however it is too variable for AMF. As previously suggested, ideal markers for fungal communities, including AMF should have high interspecific but low intraspecific variation (Lindahl et al., 2013). Thus for AMF, a more conserved region, that is of 1.5-kb long fragment comprising parts of both the large (LSU) and small subunit (SSU) rRNA genes and the complete ITS region has been proposed (Stockinger et al., 2010). This aims to provide a better resolution even at lower taxonomic ranks, i.e., species level. Moreover, the development of high-throughput sequencing

technologies such as long-read sequencing using PacBio or Nanopore platforms, can greatly increase taxonomic identification of microbial communities like AMF (Tedersoo et al., 2018; Nilsson et al., 2019). These platforms operate at the level of single DNA molecule, i.e., SMRT methodology (single-molecule real-time sequencing) and offer longer read length, require lower number of sequencing read to cover AMF diversity compared to short-read sequencing technologies and allow differentiation of closely related AMF taxa (Schlaeppi et al., 2016).

Although these marker genes are frequently used to characterize the taxonomic composition and phylogenetic diversity of specific microbial groups, these studies display several shortcomings. They focus on only one or a few universal genes and therefore it is not possible to extract accurate information of functional capabilities based on the taxonomy of all microorganisms in a complex environment like soils.

Microbial functional diversity

Ongoing advances in sequencing technologies such as high-throughput sequencing-based omics approaches allow us to obtain multiple information about the soil microorganisms. This information includes their taxonomic profile (marker-gene based sequencing), their functional potential (metagenomics) and also the active microorganisms in the soil (metatranscriptomics) (Fig. 1) (Urich et al., 2008; Prosser, 2015). Briefly, metagenomics involves the extraction of DNA from soil samples and provides information about which microorganisms are present and what their functional potential is. On the other hand, metatranscriptomics is based on the extraction of RNA, followed by the synthesis of cDNA, amplification and sequencing or direct sequencing of mRNA. Metatranscriptomics provide information on the expression status of the genes, which gives insights into what microorganisms actually do at the time of sampling (Fig. 1).

Although the amount of information yielded from these methodologies is large, these molecular tools have some limitations. Particularly, ultra-deep sequencing is needed to achieve comprehensible information on microbial diversity in a complex environment like soils. Moreover, the functional genes are diverse and form a small portion of the nucleic acid pool, which is usually the case in soils. To overcome these issues, a probe-capture enrichment technique has been developed and applied in metagenomics studies ('captured metagenomics') (Manoharan et al., 2015; Manoharan et al., 2017a). It is based on the enrichment and sequencing of several thousand functional genes of interest, like sequences coding for enzymes involved in different ecosystem functions. Studying soil microbial genes coding for specific enzymes related to an ecosystem process can enhance our understanding in describing the microbial functional potential in soils and can help us to establish a link between community structure and ecosystem functions, such as carbon cycling (Manoharan et al., 2017a). However, to date, most studies focus on the functional microbial potential, though they do not provide information about active microorganisms (mRNA). In order to understand microbial responses, RNA-based

studies are needed, however, there are many challenges when working with RNA, particularly from soils. The main obstacles are difficulties in RNA extraction due to the presence of RNases and ineffective cell lysis (Carvalhais et al., 2012). Further, the total RNA pool consists mainly of ribosomal RNA (rRNA), while mRNA is found in low amounts (1-5%) (He et al., 2007; Mettel et al., 2010). Therefore, soil samples should be quickly inactivated to prevent mRNA turnover and prior sequencing, enrichment of mRNA is required. The enrichment of mRNA could be done using enrichment of polyA-tailed mRNA or by removal of rRNA (Poretsky et al., 2005; Bailly et al., 2007). However, challenges remain as poly-A based enrichment only works for mRNA from Eukaryotes and also the rRNA removal is seldom efficient in environmental samples like soils. Hence, the above-mentioned probe-capture enrichment technique has the capability of enriching cDNA libraries obtained from reverse transcribing RNA molecules for expressed regions of interest. Based on these efficient molecular methodologies, it should be possible to better understand the functional groups of the soil microbial communities involved in different ecosystem functions (Fig. 1).

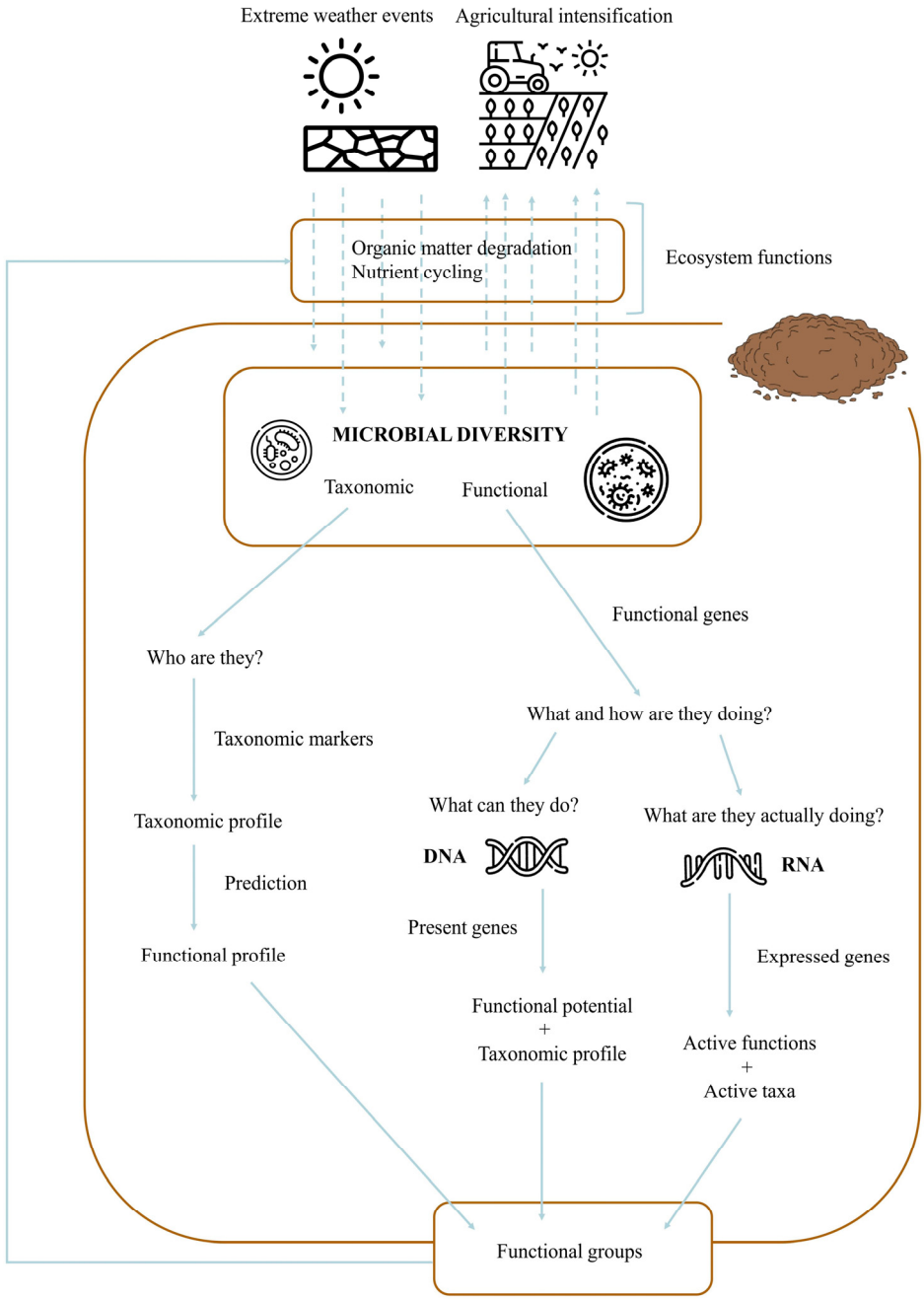

Figure 1. Schematic representation of the main strategies to study soil microbial diversity affected by the anthropogenic climate change factors.

Aims of the thesis

In this thesis, I explore how agricultural intensification and extreme weather events, specifically short-term drought can influence the taxonomic and functional diversity of soil microorganisms in agriculture. Specifically, the following aims were addressed:

- How do agricultural land-use and crop residue incorporation influence the activity of soil microorganisms involved in SOM degradation? (**Paper I**)
- How does long-term organic and conventional farming practices influence the taxonomic diversity and community composition of AMF? (**Paper II**)
- How does soil organic carbon in agricultural soils influence the functional diversity and composition of genes involved in SOM degradation? (**Paper III, IV**)
- How does short-term experimental drought influence microbial diversity and their functioning? (**Paper II, III, IV**)

Methods

The following section outlines the experimental set-ups and methodological approaches used in this thesis. A combination of field (**Paper II, III**) and glasshouse (**Paper I, IV**) experiments along with different molecular methods, specifically high-throughput sequencing-based omics approaches was used.

In **Paper I**, I studied how the expression of microbial functional genes related to SOM degradation differs between different agricultural land-uses and also how the addition of organic substrates influences their gene expression. To achieve this, the 'captured metatranscriptomics' technique, based on the sequence probe-capture enrichment technique that was previously applied in metagenomics studies ('captured metagenomics') (Manoharan et al., 2015; Manoharan et al., 2017a) was used. This technique is used to sequence a large number of genes, with the ability to enrich the genes that are either lowly abundant or expressed in the total gene pool. The enrichment of genes is done by using the custom-designed, hybridization-based oligonucleotide probes generated through a MetCap probe-designing pipeline (Kushwaha et al., 2015). This pipeline allows users to design probes for targeting functional genes involved in different microbial processes, like carbon, nitrogen and phosphorus cycling. Generated probes are hybridized with extracted nucleic acids (DNA or cDNA) and only the probe-bound nucleic acid fragments are sequenced.

Soils from two land-uses, managed as grasslands (G) and conventional farming for winter wheat (W), from Southern Sweden were collected and a glasshouse experiment was established. Experimental units (pots) were filled with fresh soils, resulting in eight soils in total (four from each land-use). Of them, in each of four pairs consisting of two land-uses, one experimental unit was used as a control, i.e., without straw addition and the second as the one to which pre-dried wheat straw was added. Throughout the one-month experimental period, bacterial and fungal growth rates using leucine and acetate incorporation methods were measured at different time points. The amount of leucine incorporated into extracted bacteria (pmol Leu incorporated g^{-1} SOM hr^{-1}) using the homogenisation/centrifugation technique (Bååth, 1992, 1994; Bååth, 2001) with few modifications (Meisner et al., 2013) was used as a proxy for bacterial growth. As a proxy for fungal growth, the amount of acetate incorporated into extracted ergosterol (pmol acetate incorporated g^{-1} SOM hr^{-1}) was used (Rousk and Bååth, 2011). Based on bacterial and fungal

responses to the straw addition, three sampling times T1, T3 and T6, corresponding to 4, 13 and 26 days after the straw addition were selected and soils were collected for the total RNA extraction. The extracted total RNA from all samples was processed for cDNA synthesis and further for the probe-capture enrichment technique. Capturing on these samples was performed using the unique oligonucleotide probes based on the gene sequences coding for enzymes responsible for the SOM degradation such as carbohydrate-active enzymes (CAZy (Cantarel et al., 2009)) and secretory proteases (MEROPS (Rawlings et al., 2012)). A local sequence database of the selected nucleotide sequences of these genes used for designing the capture-probes, here called targeted database (TDB) was set-up. Captured cDNA libraries were prepared as described by Manoharan et al. (2017a) and sequenced on a single lane of the Illumina HiSeq 2000 system (paired-end mode, 125bp read length). Finally, the gene expression in different land-uses and after the addition of organic substrates was determined based on the annotations matching the TDB.

In **Paper II**, I tested the effects of a short-term experimental drought on the diversity and community composition of AMF in organic and conventional farming systems. The experiment was conducted within the DOK (bio**d**ynamic, bio**o**rganic and conventional [**k**onventionell]) long-term agricultural experiment (Therwil, Switzerland) (Fig. 2) that compares organic and conventional farming systems. These systems differ in fertilization and plant protection practices but follow the same seven-year crop rotation (Mäder et al., 2002; Krause et al., 2020).

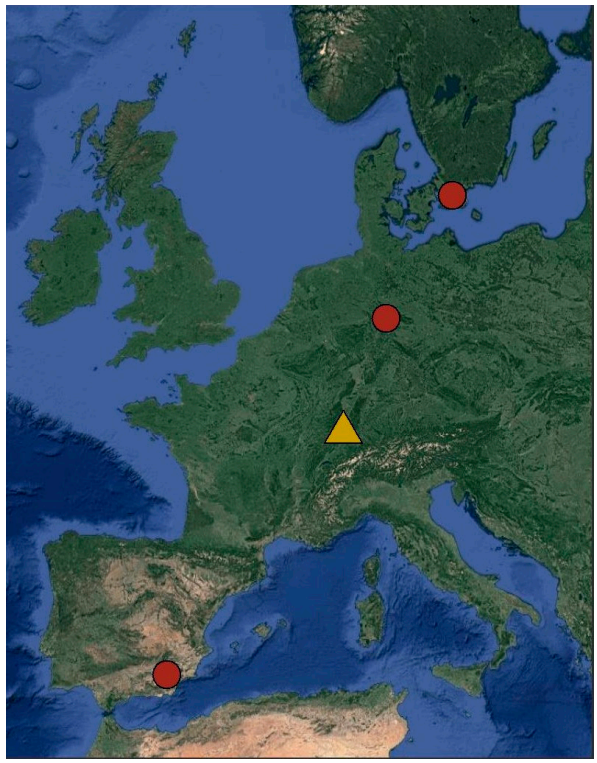

Figure 2. Overview of the agricultural areas across Europe in **Paper II** and **III**. The triangle (yellow colour) marks the DOK (bio**d**ynamic, bio**o**rganic and conventional [**k**onventionell]) long-term agricultural experiment in Switzerland. Three circles (brown colour) mark the three agricultural areas ranging from Northern to Central and Mediterranean Europe (Sweden, Germany and Spain). In **Paper IV** soils from the agricultural areas of Sweden and Spain were used.

In two farming systems, biodynamic (BIODYN) and conventional (CONMIN), a drought manipulation experiment with rainout-shelters to impose a drought by reducing the ambient precipitation (Kundel et al., 2018) was established in mid-March and ended in June 2017. To determine the drought effects by the fixed location rainout-shelters the three drought treatments consisted of: I) a rainout-shelter reducing 65% of the precipitation (Roof treatment, R, Fig. 3), II) a control treatment with modified rainout-shelter to quantify potential rainout-shelters artefacts (Roof-Control treatment, RC), and III) an unmanipulated control without a rainout shelter (Control treatment, C). Throughout the spring and summer winter wheat (*Triticum aestivum* L., cv. Wiwa) growing season, soil samples for the DNA extractions were collected at two occasions: 4 and 13 weeks after the rainout-shelter establishment. To describe AMF communities, 1.5-kb long fragment of the nuclear rRNA gene, comprising the entire ITS, parts of SSU and LSU subunit (Kruger et al., 2009) was amplified using the two-step PCR protocol. These AMF amplicons were sequenced using the SMRT methodology (PacBio). From the raw sequencing reads, circular consensus sequences (CCS) were generated and clustered into amplicon sequence variants (ASVs).

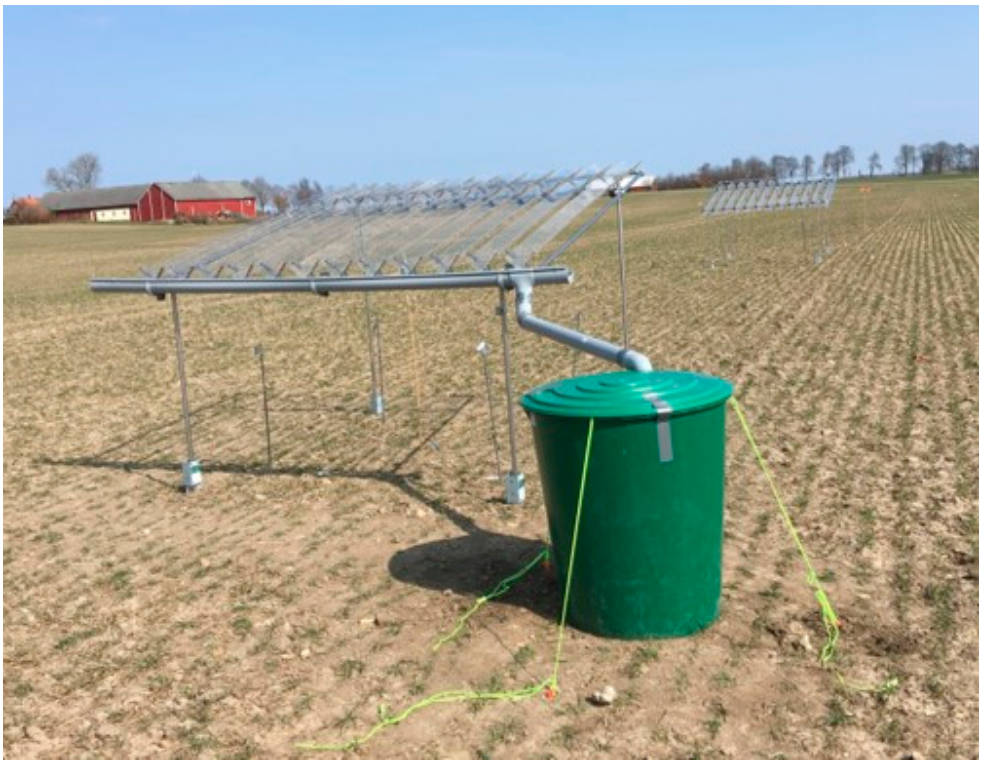

Figure 3: Drought manipulation experiment in **Paper II** and **III**. Fixed location, partial rainout-shelters to impose a drought by reducing the 65% of the precipitation (Roof treatment, R) across the spring and summer winter wheat growing season. Photo: María Ingimarsdóttir.

In **Paper III**, I studied how short-term experimental drought and contrasting SOC levels influence the diversity and composition of functional genes in agricultural soils over a range of different climatic conditions and soil properties.

An agricultural experiment was set-up across Europe (Fig. 2), ranging from Northern to Central and Mediterranean Europe. In three agricultural areas; in Southern Sweden (region Scania: SE), Northwestern Germany (region Lower Saxony: DE) and Southeastern Spain (region Almería: ES) agricultural fields with contrasting levels of SOC (i.e., "low" (~1%) and "high" (~3%) categories) were selected and the three drought treatments (as in **Paper II**, Fig. 3) installed. At the mature stage of winter wheat, soil samples for DNA extractions were collected and the 'captured metagenomics' technique (Manoharan et al., 2015) was applied to the extracted DNA. A probe-capture enrichment technique used in this study was similar to **Paper I** but capturing the functional genes coding only for extracellular enzymes responsible for the degradation of SOM. Unique oligonucleotide probes designed based on the genes coding for carbohydrate-active enzymes (CAZy) with excretory signal peptides were used for the enrichment. Based on these selected

nucleotide sequences a database; exTDB - extracellular targeted database was set-up. Soil DNA libraries were hybridized with custom-designed oligonucleotide probes, and finally captured DNA libraries were sequenced (paired-end mode, 150 bp read length) on Illumina HiSeq 4000 system. Functional genetic diversity, the functional and taxonomic gene composition were evaluated based on the annotations matching the exTDB.

To identify microbial responses to short-term experimental drought in agricultural soils, a glasshouse experiment was established in **Paper IV**.

Soils from two agricultural areas (Fig. 2), i.e., Southern Sweden (region Scania: SE), and Southeastern Spain (region Almería: ES) with the most contrasting soil properties (e.g., SOC content, soil pH, soil texture) exposed to different climatic conditions from **Paper III** were chosen. Experimental units (pots) were filled with fresh soils and by manipulating soil water content, i.e., 60% of water holding capacity (WHC) (control) or 30% WHC (drought) a short-term experimental drought was established and maintained throughout the barley (*Hordeum vulgare* cv Bonus) growing period (Fig. 4). During the drought experimental period (eight weeks in total), soils were sampled every second week (five sampling occasions in total) and used for different measurements. For example, microbial respiration, a measure of the metabolic activity of the soil microorganisms was measured. In addition, soil samples for the analysis of phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) used for biomass estimates of bacteria and fungi (PLFAs) and AMF (NLFAs) were also collected. To identify how gene expression is affected by short-term experimental drought, soil samples were collected at two sampling occasions, before the drought period started (T0; zero weeks) and at the end of the drought period (T4; eight weeks). In total, at each of these two sampling occasions, 20 soil samples were collected, resulting in a total of 40 soil samples for the total RNA extraction. From the total RNA extracts, libraries were prepared and subjected to sequencing on NovaSeq 6000 System (paired-end mode, 150 bp read length). The obtained sequence reads were screened for rRNA, and only non-rRNA sequences were then mapped (in-silico) to the exTDB that was established in **Paper III**.

Figure 4: Glasshouse drought manipulation experiment in **Paper IV**, at the beginning of the drought experimental period (left picture) and at the end of the drought experimental period (right picture). Photo: Katja Kozjek.

Results and discussion

Effects of agricultural land-use intensification

Land-use effects

Land-use practices have been identified to alter soil microbial community composition (Jangid et al., 2008; Kaiser et al., 2016; Madegwa and Uchida, 2021), their functional genetic diversity (Manoharan et al., 2017a), and also their gene expression (Nacke et al., 2014). As expected, in **Paper I** the overall expression of genes related to organic matter degradation was mainly affected by their land-use practices (grassland and agricultural soils). Grassland soils were similar, while agricultural soils displayed higher variability in their gene expression (Fig. 5). These differences could be explained by soil disturbances (physical and chemical) caused by land-use practices. Agricultural soils are commonly exposed to tillage and crop protection strategies, i.e., the application of fertilizers (Madegwa and Uchida, 2021; Yang et al., 2021), while grasslands remain undisturbed. Further, in **Paper I**, it was found that the diversity of differentially expressed genes in agricultural soils was higher compared to grasslands. This difference was further enhanced by the straw addition. The majority of differentially expressed genes in agricultural soils belonged to the families of the GH class, known for the degradation of multiple substrates like cellulose and hemicellulose (Henrissat and Davies, 1997). Many differentially expressed genes in grasslands also belonged to the families of the GH class. However, the majority of differentially expressed genes belonged to the GT class, especially enzyme family GT2, even after the straw addition. This suggests that many of these genes in grasslands were involved in the biosynthesis of carbohydrate molecules rather than their degradation as observed in agricultural soils.

In contrast to grasslands, amounts of SOC in agricultural soils are generally lower, moreover agricultural soils are under considerable risk due to practices like tillage and intensive fertilization. They negatively influence soil quality, including losses of SOC (Lal, 2013). In **Paper I**, enzymes CDA1 and XynA were highly differentially expressed in grasslands while comparing control samples of both land-uses. However, these two enzymes were then highly promoted in agricultural soils when straw was added. Downregulation of these enzymes and upregulation of the

above-mentioned GTs due to straw addition in grasslands suggest that straw addition led to carbon storage or sequestration. However, the straw in SOC poor agricultural soils was used as a source of carbon and energy. Altogether, the addition of straw triggered microbial responses similar to grasslands which indicates that with careful land-use practices agricultural soils have the potential to become more productive.

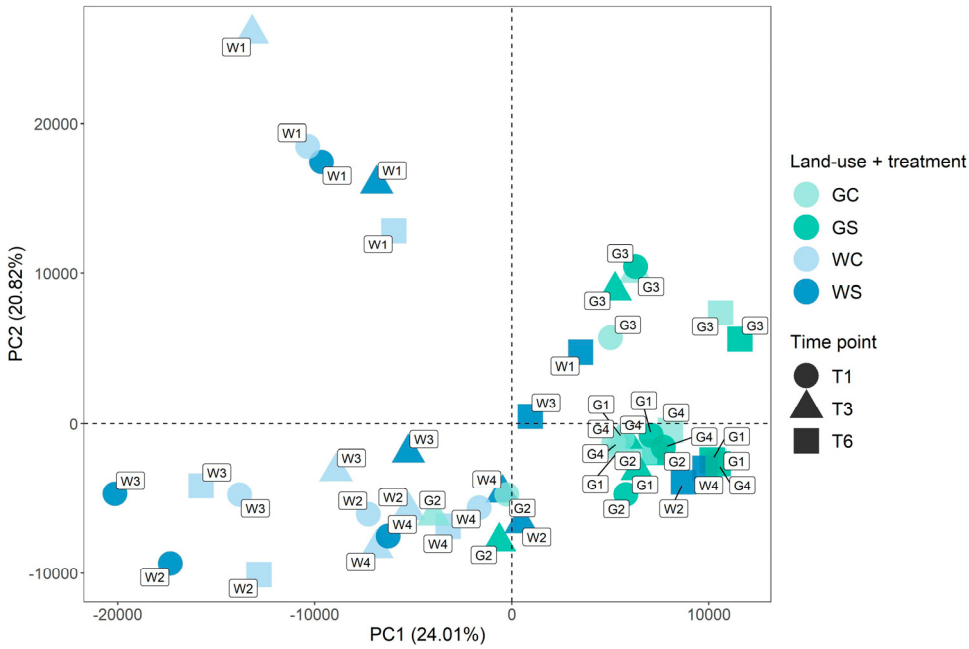

Figure 5. Principal component analysis of the TMM normalized counts. Samples are categorized by land-use and treatment condition (as colour) and sampling time (as shape). Text labels represent the location of each field (W – winter wheat, G – grasslands). Abbreviations: GC – grasslands without straw addition; GS – grasslands with straw addition; WC – winter wheat without straw addition; WS – winter wheat with straw addition (**Paper I**).

Crop residue incorporation

Paper I reported a promoted microbial activity and gene expression across both land-uses when fresh organic material, i.e., wheat straw was added to grassland and agricultural soils. Due to low SOC content in agricultural soils and consequent starvation of microorganisms, the addition of straw that contains a high amount of organic carbon (Liu et al., 2014), particularly enhanced gene expression in agricultural soils compared to grasslands. Furthermore, with time, even though the gene expression patterns among agricultural soils differed, the straw addition made them similar to that of grassland soils. These findings together with previous literature show that improved crop residue management has the potential to promote SOC levels in agricultural soils (Lessmann et al., 2021) and promote the functioning of soil microorganisms, especially their carbon cycling genes.

Straw addition mainly upregulated different enzyme families in agricultural soils compared to grasslands, where there were also many enzyme families that were downregulated. Enzyme families GH13 and GH23 were the major ones that were upregulated by the straw addition in both land-uses (Fig. 6). Typically GH13 acts on multiple carbohydrate substrates (Stam et al., 2006) while GH23 degrades peptidoglycan and chitin (Scheurwater et al., 2008; Liao et al., 2019). As mentioned earlier, many genes that belonged to these two enzyme families were downregulated by the straw addition especially in grasslands (Fig. 6b). Genes of enzyme family GH43 that degrade hemicellulose (Mewis et al., 2016) were upregulated by straw addition in both land-uses but were especially highly upregulated in grasslands (Fig. 6b). Whereas enzyme families like GH6, 8 and 9 that are mainly characterized with cellulolytic activities (Brumm, 2013) were highly upregulated in agricultural soils (Fig. 6a).

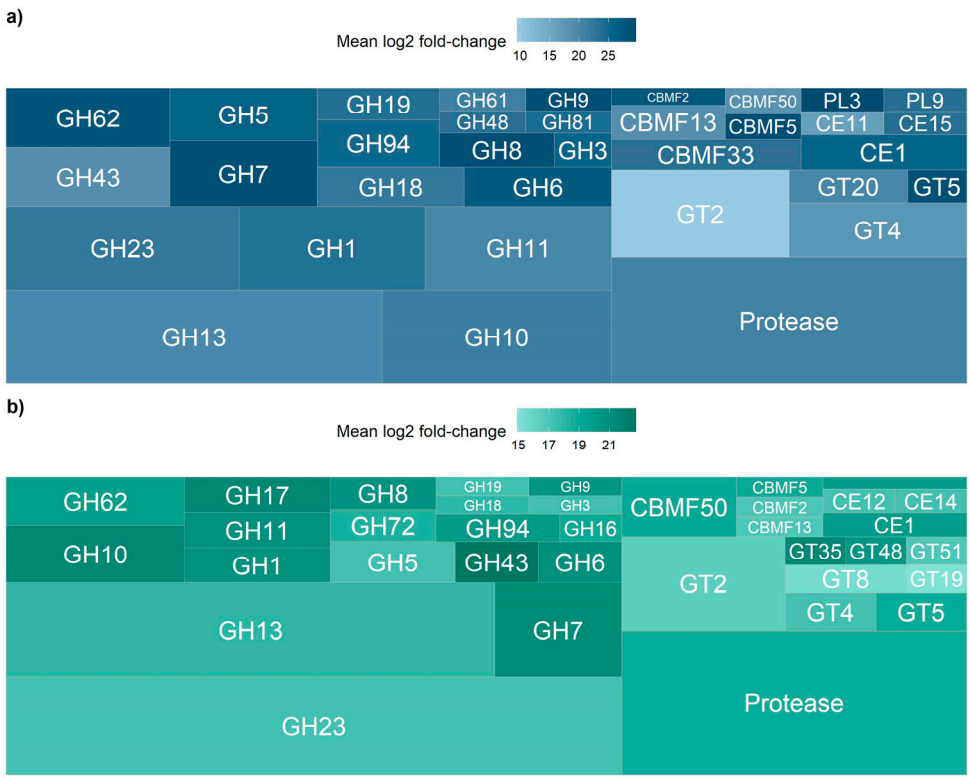

Figure 6. The significantly upregulated transcripts by the straw addition in wheat (a) and grassland soils (b). Each box represents a CAZy or the protease enzyme family with their area representing the number of differentially expressed transcripts and the colour gradient represents their mean fold-change values of those transcripts. The boxes are sorted based on each enzyme family's area and also the enzyme class that they belong to. Abbreviations: auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyltransferases (GT), and polysaccharide lyases (PL) (**Paper I**).

Farming systems

The effects of different farming practices, such as organic or conventional farming on soil microbial communities have been widely studied (Xue et al., 2013; Hartmann et al., 2015; Lori et al., 2017). However, there are contrasting results of how different farming practices influence communities of AMF. Results in **Paper II** showed that long-term organic (biodynamic) and conventional farming systems did not affect the diversity of these important root-associated fungal communities, while the AMF community composition was significantly affected (Fig. 7). Contrary to expectations, organic farming did not promote the diversity of AMF. In line with previous findings (Birkhofer et al., 2012; Xiang et al., 2014; Manoharan et al., 2017b), AMF community composition differed significantly between the farming systems and also across the growing season of winter wheat (Fig. 7). Taken together, several lines of evidence highlight changes in the community composition of AMF in contrast to AMF diversity due to different farming systems. This suggests that the same taxa are present across the farming systems with shifts in abundance rather than AMF diversity changes.

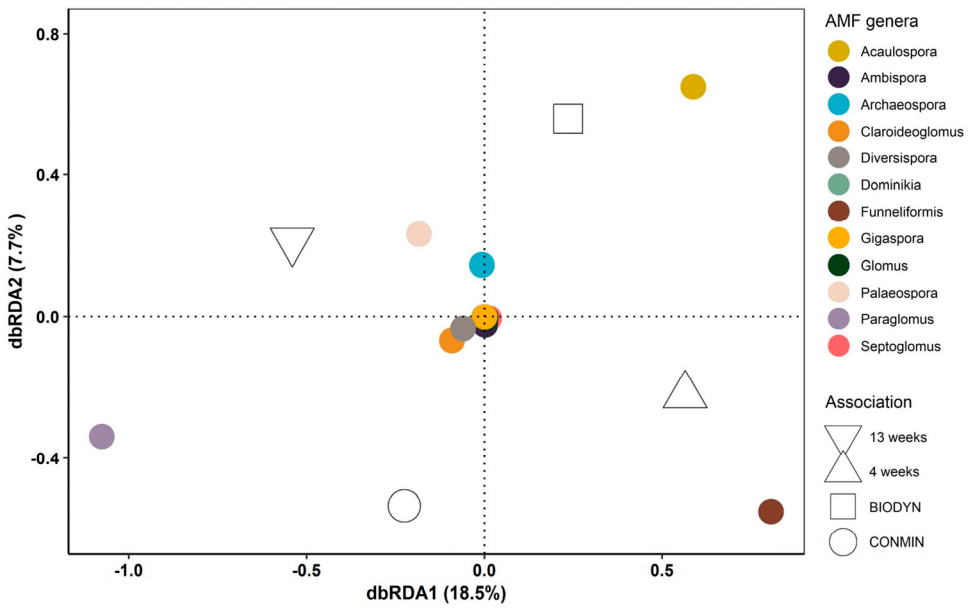

Figure 7. Distance-based redundancy analysis (db-RDA) of arbuscular mycorrhizal fungal (AMF) community composition at the genus level, constrained for the farming systems and the sampling time. The different shapes represent the association with one of the farming systems (BIODYN, CONMIN), and the sampling times (4 and 13 weeks after the rainout-shelter establishment) and colours represent the AMF genera (**Paper II**).

Effects of short-term drought on soil microbial diversity

Agricultural land-use intensification in combination with increased occurrence of extreme weather events such as drought, could impact soil microbial communities, their diversity and functioning and consequently limit their capacity to provide crucial ecosystem services to humanity. Negative effects of drought on soil microorganisms were reported (Zhou et al., 2020), however the studies addressing these effects on the taxonomic and functional diversity at a high resolution in agricultural soils are limited. This thesis (**Paper II, III** and **IV**) provides more insights on how the above-mentioned threat influenced the taxonomic and functional diversity of soil microbial communities.

Effects on the taxonomic diversity

The short-term experimental drought did not influence the AMF diversity or their community composition **(Paper II)**. The resistance of AMF to drought periods is probably due to their filamentous structures with the ability to form complex hyphal networks which allow them to survive in environments with limited water availability (Allen, 2007; Manzoni et al., 2012). An alternative explanation for AMF communities being able to cope well with short-term drought could be high SOC content in these soils. Generally, high levels of SOC can improve the soil quality, enhancing soil infiltration and soil water retention (Rawls et al., 2003; Lal, 2016) and can potentially act as a buffer to drought effects (Lal et al., 2011). SOC content in both farming systems influenced soil water content, however it was not possible to conclude if high levels of SOC had a direct influence on the AMF communities. To better understand if SOC content has beneficial effects on AMF communities under drought, factors influencing SOC dynamics in soils should be studied separately. Although no effects of the short-term experimental drought on AMF communities were detected, some AMF taxa were identified as indicators for drought conditions. Particularly, we found drought indicator taxa represented by ASVs in the family Archaeosporaceae. So far only a few studies have investigated AMF responses to drought and have found *Glomus* and *Diversispora* species (Yang et al., 2010; Zhang et al., 2016; Deveautour et al., 2018) being tolerant to drought. Thus, it remains to be further explored if fungi from the family Archaeosporaceae are particularly resistant to drought conditions.

Further, in **Paper II**, the identification of AMF communities at a fine level of resolution, the ASV level, displayed some shortcomings. AMF have a high intraspecific genetic variation, within a species and also within a single spore (Sanders, 2004). Each ASV represents a unique DNA sequence of on organism (Callahan et al., 2017; Glassman and Martiny, 2018), here AMF taxa, but since current taxonomic databases lack detailed information on AMF intra- and interspecific variation, assignment of AMF ASVs to a genus or species level is

challenging. Altogether, these observations along with the challenge to infer microbial functions from taxonomy highlight the need to study the microbial functional genes. That way it is possible to better understand the behaviour of soil microorganisms.

Moreover, based on the taxonomy obtained from the functional genes in **Paper III**, the short-term experimental drought did not have an impact on the taxonomic composition of the microbial communities in soils similar to **Paper II**. In both cases, taxonomic compositions were mainly explained by their regional differences, i.e., country of origin. When comparing soils between countries in **Paper III**, compared to Spain, Proteobacteria was more abundant in Sweden and Germany (SOC rich soils) while in Spain (SOC poor soils) Actinobacteria was prevalent. This trend was also observed in **Paper I**, where Actinobacteria was more active in SOC poor agricultural soils compared to SOC rich grassland soils, where Proteobacteria was more active. When straw was added to agricultural soils the abundance of Actinobacteria started diminishing. In **Paper IV**, Proteobacteria was downregulated by drought in SOC rich soils of Sweden, while Actinobacteria was upregulated by drought. Based on findings from **Paper I**, **III** and **IV** and previous studies (Bouskill et al., 2013; Mohammadipanah and Wink, 2016; Canarini et al., 2021), it is clear that Actinobacteria are more resistant to disturbances (nutrient or water availability) in soils compared to Proteobacteria. However, it is important to keep in mind that there were some Proteobacteria upregulated by drought that could potentially be specialists (**Paper IV**) (Spain et al., 2009).

Effects on the functional diversity

The drought effects on soil microorganisms have primarily been addressed to study changes in community composition and taxonomic diversity (Bouskill et al., 2013; Ochoa-Hueso et al., 2018; Canarini et al., 2021). In **Paper III** and **IV**, we investigated the effects of drought on soil microbial communities at their functional genetic level, specifically on their ability to degrade SOM. The diversity and composition of genes encoding for extracellular enzymes were determined on the DNA level in **Paper III**. Whereas in **Paper IV**, the expression of genes from **Paper III** was analysed on the RNA level, based on the glasshouse experiment simulating drought.

While there were differences in their functional genetic potential to degrade SOM between the three European agricultural areas, Sweden, Germany and Sweden, the drought effect was only observed within agricultural fields in Germany (**Paper III**). The functional genetic composition in Sweden and Spain seemed resistant to short-term drought (**Paper III**), however drought affected the microbial responses in their ability to degrade SOM (**Paper IV**). Resistance to short-term drought in Spanish soils based on their functional genetic potential (**Paper III**) might be explained by the regional adaptations of soil microorganisms to already dry environments, such

as arid and semiarid environments. Soil microorganisms in these environments developed different strategies to survive drought periods, such as thicker cell walls, ability to form spores, accumulation of osmolytes or production of extracellular polymeric substances (Schimel, 2018). However, it was interesting to note that the diversity of these enzymes in Spanish soils was higher compared to Swedish and German soils (Fig. 8.). On the other hand, the resistance to drought in Swedish soils could be explained by the extreme drought of the experimental year (Di Liberto, 2018) and the potential buffering ability of SOC (Lal et al., 2011). In contrast to Spain and Germany, Swedish soils contained high levels of SOC, therefore the drought effect on the functional gene composition might be overshadowed by higher SOC levels. Most likely high levels of SOC buffered the soil water levels to a degree that made the composition of functional genes resistant to water shortages.

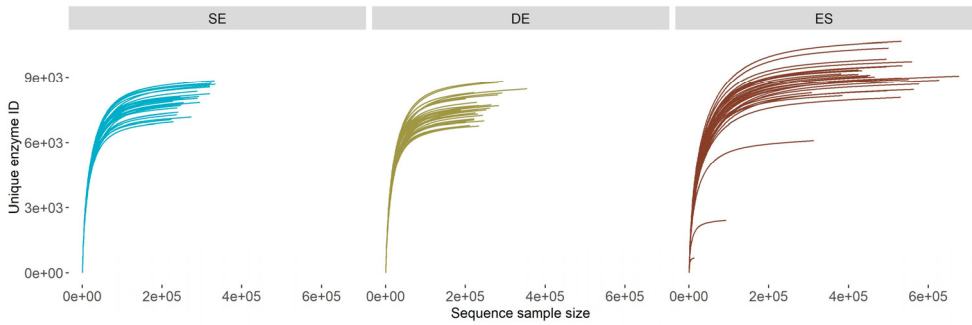

Figure 8. Rarefaction curves of the unique enzyme IDs obtained from random sampling of on-targets from the DIAMOND blastx matches against exTDB in the three countries (SE – Sweden, DE – Germany and ES – Spain). The x-axis represents the number of sequence reads, and the y-axis represents the number of captured unique enzyme IDs (**Paper III**).

The drought resistance of microbial communities in Spain was even more evident when their response to drought on the RNA level was studied (**Paper IV**). The number of differentially expressed genes due to drought was much lower in Spain compared to Sweden (Fig. 9). Although the diversity of enzymes on the gene level in Spain was the highest (**Paper III**, Fig. 8), the diversity of expressed enzymes affected by the drought was much lower than in Sweden.

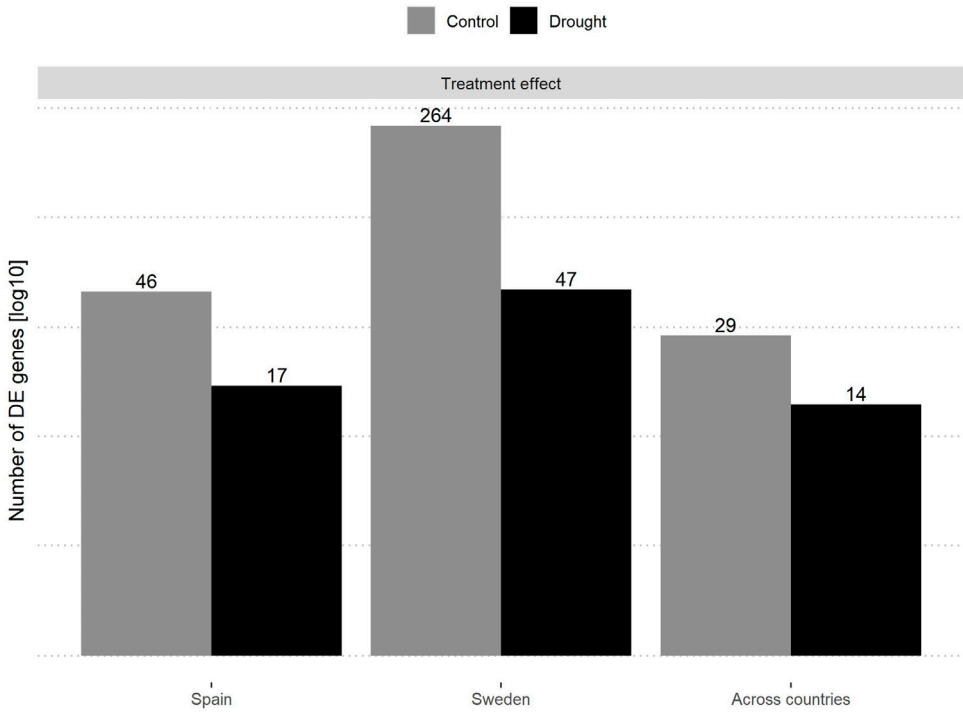

Figure 9. Bar plot showing the number of significantly (padj < 0.05) differentially expressed (DE) genes up- (drought) and down- (control) regulated by the treatment in each country (Spain and Sweden). The y-axis is scaled at log10 for visualization (**Paper IV**).

Links between taxonomic groups and functions

One of the main advantages of studying functional genes, present or expressed, is the ability to obtain information of the microbial taxonomic composition and their functional capabilities simultaneously. While studies focusing on only taxonomic diversity are primarily limited to describing taxonomic changes. In this thesis, links between microbial taxonomic groups and functional genes helped to better understand the microbial mechanisms behind SOM degradation.

Class AA, a key class for the decomposition of lignin (Lombard et al., 2014), has been found in **Paper III**, and **IV**. More specifically, on the DNA level (**Paper III**), enzyme family AA10 has been found in all three countries and linked to Actinobacteria. However, this enzyme family was evidently more abundant in drier and SOC depleted soils of Spain. It was not surprising that this enzyme family was found in high abundance in these soils, because Actinobacteria are adapted to these harsher conditions (Acosta-Martínez et al., 2014; Mohammadipanah and Wink,

2016). On the other hand, on the RNA level (**Paper IV**), in the same soils, this enzyme family has been found to be promoted by the drought but linked to Proteobacteria. Therefore, this enzyme family could be a specialist for these soil types (Spain et al., 2009; Islam et al., 2020). In Swedish soils, AA10 was also promoted by the drought and found in Actinobacteria. Since AA10 is responsible for the degradation of complex carbohydrates and has been promoted by the drought in both countries, these results may indicate that due to low water availability simpler sugar molecules were not accessible and microorganisms were forced to search for complex carbohydrates. The enzyme family CBM50 was upregulated by straw addition in both land-uses (**Paper I**, Fig. 10), and mainly from Actinobacteria in agricultural soils and from Proteobacteria and Firmicutes in grasslands. In **Paper III**, on the DNA level, CBM50 was negatively correlated to SOC. In Sweden and Spain CBM50 was found in Actinobacteria and Proteobacteria, while in Germany this enzyme family was only of proteobacterial origin. Interestingly drought upregulated this enzyme family only from Proteobacteria (**Paper IV**). Observation on CBM50 is similar to that of AA10 in the sense that Actinobacteria are generally more adapted to drier and SOC depleted soils (Mohammadipanah and Wink, 2016), while there might be some Proteobacteria adapted to drought conditions (Spain et al., 2009). Among other CBM families, CBM13 has been found in **Paper I**, **III** and **IV** and linked to Actinobacteria. Interestingly, drought upregulated only Actinobacteria in Swedish soils, while it was only downregulated in Spanish soils (**Paper IV**). Actinobacteria perform well in dry soils (Acosta-Martínez et al., 2014; Maestre et al., 2015), but it might also be that the drought did not differentially affect Actinobacteria in Spanish soils. The same trend was also observed for CBM2.

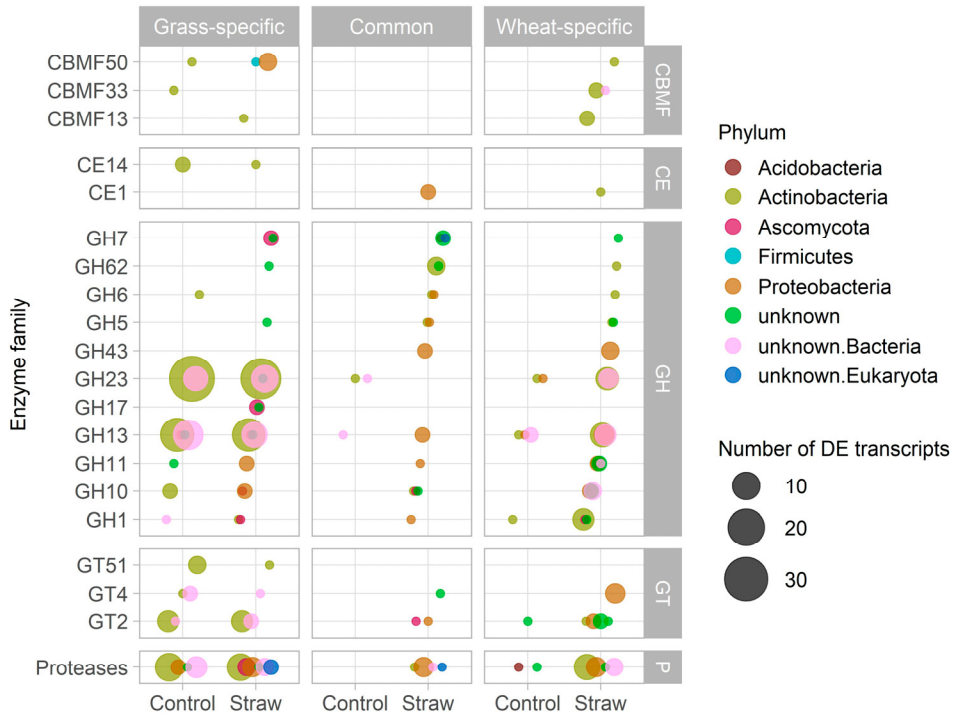

Figure 10. The differentially expressed (DE) transcripts by the straw addition in both land-uses. These DE transcripts were sorted based on if they were common or specific to a particular land-use and also if they were up- (straw) or downregulated (control) by the straw addition (x-axis). Further they were sorted based on the CAZy or protease enzyme family they belong to (y-axis). The size of each point represents the number of DE transcripts in each category and the colour represents their taxonomy at the phylum level. Abbreviations: auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), and proteases (P) (**Paper I**).

Families CE1 and CE4 were positively linked to SOC on the DNA level (**Paper III**) and were predominantly downregulated by drought in Sweden and Spain (**Paper IV**). Limited water availability upregulated Actinobacteria and Ascomycota of CE1 in Sweden (**Paper IV**). These findings suggest that higher levels of SOC content could act as a buffer to drought by promoting upregulation of actinobacterial CE1 (Lal et al., 2011). Links between CE1 and SOC were even more evident in **Paper I** (Fig. 10), as they were upregulated by the addition of straw in both land-uses. CE4 was strongly linked to Proteobacteria in Swedish and German soils, while in Spain it was mostly linked to Actinobacteria (**Paper III**). Proteobacteria, Firmicutes, Actinobacteria and Ascomycota harbouring CE4 were downregulated by drought in Swedish soils, while Firmicutes were the only phylum that was upregulated by drought in these soils (**Paper IV**). In line with previous studies (Bouskill et al., 2013; Hartmann et al., 2017) Firmicutes are resistant to desiccation and can perform well in dry environments, therefore not surprising they were upregulated by drought in Sweden (**Paper IV**). Among GTs, GT2 was found to be upregulated by straw in

grasslands and agricultural soils, however found in different taxonomic groups. In grasslands mainly in Actinobacteria and in agricultural soils mainly in Proteobacteria (**Paper I**, Fig. 10). Drought did not upregulate any specific GT2, while downregulation in Spanish soils was found for Firmicutes and Proteobacteria, and Proteobacteria and Acidobacteria in Swedish soils (**Paper IV**). As found in **Paper I** (Fig. 10), proteobacterial GT4 was strongly upregulated by straw addition in agricultural soils. Enzyme GT51 was negatively correlated to SOC (**Paper III**) but downregulated by the drought in Spain and Sweden (**Paper IV**). Moreover, the addition of straw downregulated most GT51 of actinobacterial origin in grasslands, while there was no effect on agricultural soils (**Paper I**, Fig. 10). As discussed earlier, GT class is mainly involved in building carbohydrate molecules (Schmid et al., 2016) and as expected enzyme families from this class were mainly upregulated by the addition of organic material but were downregulated by drought. In many GH families, such as GH7, 17, 10 and 1 Ascomycota was found to be upregulated by straw addition, but only in grasslands (**Paper I**, Fig. 10). Interestingly, none of these enzyme families was found in **Paper III** and **IV**. It is also worth mentioning families GH13 and GH23, as they were both up- and downregulated by straw addition in both land-uses, but strongly in grasslands (**Paper I**, Fig. 10). Across all three European countries, GH13 was linked to Proteobacteria in Sweden and Germany, and to Actinobacteria and Proteobacteria in Spain (**Paper III**). Drought upregulated actinobacterial GH13 in Sweden, while proteobacterial GH13 was upregulated in both countries. In the case of GH23, Sweden and Germany shared the same trend, with Actinobacteria and Proteobacteria in similar proportions, while in Spain the proportion of Actinobacteria compared to Proteobacteria was higher. In contrast to Sweden, where none of the GH23 taxa was upregulated by drought, but Proteobacteria was upregulated by drought in Spain. However, both Actinobacteria and Proteobacteria were upregulated by drought across both countries (**Paper IV**).

Conclusions and future perspectives

Functional diversity of soil microorganisms is the essential link between soil microbial groups and ecosystem functions, which provides new insights in understanding soil biodiversity. There is an increasing recognition that in contrast to taxonomic information and inferring functions from taxonomy, functional diversity may provide a more comprehensive understanding of the functioning of microbial systems (Escalas et al., 2019). The advantage of studying functional genes, present or expressed, can enable the identification of soil functional groups in the ecosystem.

This thesis gives further insights into how agricultural land-use intensification and extreme weather events, especially drought affect soil microbial communities, their taxonomic and functional diversity and how this is linked to ecosystem processes. In this sense, **Paper I** showed that different land-use types altered microbial responses, particularly in relation to SOM degradation. Then, it was further explored how different agricultural practices could prevent traditionally carbon depleted agricultural soils from becoming poorly productive in the long run and if some of these practices have the potential to mitigate the effects of extreme drought (**Paper I**, and **II**). **Paper I** showed that the addition of organic compounds enhanced the expression of carbon cycling genes, particularly in agricultural soils. Organic farming did not promote the diversity of AMF communities, however high levels of SOC in this farming system may have a positive effect on the resistance of AMF communities against drought (**Paper II**). Taxonomic diversity of soil microbial communities, specifically AMF was not affected by drought (**Paper II**), while functional gene composition across Europe was affected in one out of the three countries (**Paper III**). These results suggest that soil microbial communities respond differently to short-term drought due to (a) their structure, like filamentous structures and hyphal networks (**Paper II**), (b) regional adaptations to survive harsh conditions in dry environments (**Paper III**), (c) differences in soil physicochemical properties, like SOC (**Paper II** and **III**). Even though some microbial communities across Europe displayed higher resistance to drought (**Paper III**), results of **Paper IV** clearly showed that short-term drought affected expressed genes involved in the degradation of SOM. Taken together, results in this thesis suggest that careful agricultural practices, including crop residue incorporation, specific farming systems and increased levels of SOC have the potential to mitigate the effects of drought on soil microorganisms and can lead towards more sustainable agriculture.

However, the results and observations in this thesis opened several interesting questions that could be addressed in the future.

For example, short-term experimental drought in **Paper II**, **III** and **IV** influenced soil water content, but soil microbial communities displayed a certain degree of resistance. As European and global climate models forecast higher occurrence and intensity of short-term drought periods (Masson-Delmotte, 2021), the verification of these results under even more extreme and repeated drought conditions should be studied. Additionally, microbial resistance to drought conditions should be further explored under an extended range of environmental conditions, including both, soil properties and climate conditions.

In **Paper II** the potential of AMF to mitigate drought effects was found. However, it would be important to include plants, specifically roots in these studies, because AMF colonizes plant roots and helps them to obtain water and nutrients from places that roots would not be able to reach. That way it would be possible to unlock the potential of AMF communities to protect and help plants survive drought periods. Such knowledge may contribute to the development of sustainable land-use systems to remain productive in long-run and maximize crop yields even under extreme drought periods.

There is potential in further using the collected data in this thesis, specifically metagenomics and metatranscriptomics from **Paper I**, **III** and **IV**. This information could be used in the integrative analysis, the so-called multi-omics as these data was obtained from the same soils. This approach may provide more comprehensive insights into soil microbial systems, both short- and long-term, and how these systems are influenced by different factors, either climate change or direct human activities. For example, the results from Manoharan et al. (2017a) showed land-use effects on the soil microbial DNA could be linked to their RNA from **Paper I** where straw was added. This way we could understand the long-term effects of land-use on the short-term effects of straw addition at a higher resolution. Similarly, linking results from **Paper III** and **IV** will provide a better understanding of how short-term drought periods could affect soil microbial communities involved in the degradation of SOM.

New sequencing technologies lead to larger amounts of high-quality data, with the potential to study even more specific microbial functions and increase the taxonomic resolution of soil microbial communities. This may further lead to the expansion of current references databases, for example fungal databases. This could (a) offer a better representation of fungal communities, which was limited in **Paper I**, **III** and **IV**, (b) help to better understand the intraspecific variation of AMF communities highlighted in **Paper II**. Captured approaches (metagenomics or metatranscriptomics) used in this thesis (**Paper I**, **III** and **IV**) present a way forward to better understand the functioning of complex soil ecosystems, especially carbon cycling. However, to have a better representation of fungi, who are important players in the degradation of SOM (Magdoff and Weil, 2004), a more specific probe-capture enrichment technique targeting only fungi could be used.

References

Acosta-Martínez, V., Cotton, J., Gardner, T., Moore-Kucera, J., Zak, J., Wester, D., Cox, S., 2014. Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. Applied Soil Ecology 84, 69-82. https://doi.org/10.1016/j.apsoil.2014.06.005

Allen, M.F., 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone Journal 6, 291-297.

Bååth, E., 1992. Thymidine incorporation into macromolecules of bacteria extracted from soil by homogenization-centrifugation. Soil Biology and Biochemistry 24, 1157-1165.

Bååth, E., 1994. Measurement of protein synthesis by soil bacterial assemblages with the leucine incorporation technique. Biology and Fertility of Soils 17, 147-153.

Bååth, E., 2001. Estimation of fungal growth rates in soil using 14C-acetate incorporation into ergosterol. Soil Biology and Biochemistry 33, 2011-2018. https://doi.org/10.1016/S0038-0717(01)00137-7

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Polme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233-237. 10.1038/s41586-018-0386-6

Bailly, J., Fraissinet-Tachet, L., Verner, M.-C., Debaud, J.-C., Lemaire, M., Wésolowski-Louvel, M., Marmeisse, R., 2007. Soil eukaryotic functional diversity, a metatranscriptomic approach. The Isme Journal 1, 632-642. 10.1038/ismej.2007.68

Barberán, A., Caceres Velazquez, H., Jones, S., Fierer, N., 2017. Hiding in plain sight: mining bacterial species records for phenotypic trait information. mSphere 2, e00237-00217.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505-511. 10.1038/nature13855

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47, 151-163.

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. Trends Ecol Evol 31, 440-452. 10.1016/j.tree.2016.02.016

Birkhofer, K., Bezemer, T.M., Hedlund, K., Setälä, H., 2012. Community composition of soil organisms under different wheat farming systems. Microbial ecology in sustainable agroecosystems, 89-111.

Birkhofer, K., Smith, H.G., Rundlöf, M., 2016. Environmental impacts of organic farming. eLS, 1-7.

Bolinder, M.A., Crotty, F., Elsen, A., Frac, M., Kismányoky, T., Lipiec, J., Tits, M., Tóth, Z., Kätterer, T., 2020. The effect of crop residues, cover crops, manures and nitrogen fertilization on soil organic carbon changes in agroecosystems: a synthesis of reviews. Mitigation and Adaptation Strategies for Global Change 25, 929-952. 10.1007/s11027-020-09916-3

Bouskill, N.J., Lim, H.C., Borglin, S., Salve, R., Wood, T.E., Silver, W.L., Brodie, E.L., 2013. Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. The Isme Journal 7, 384-394. 10.1038/ismej.2012.113

Brumm, P.J., 2013. Bacterial genomes: what they teach us about cellulose degradation. Biofuels 4, 669-681. 10.4155/bfs.13.44

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58, 216-234. 10.1016/j.soilbio.2012.11.009

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11, 2639-2643. 10.1038/ismej.2017.119

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C.W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M., Richter, A., 2021. Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. Nature Communications 12, 5308. 10.1038/s41467-021-25675-4

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37, D233-238. 10.1093/nar/gkn663

Carvalhais, L.C., Dennis, P.G., Tyson, G.W., Schenk, P.M., 2012. Application of metatranscriptomics to soil environments. J Microbiol Methods 91, 246-251. 10.1016/j.mimet.2012.08.011

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J., Hutchins, D.A., Jansson, J.K., Karl, D.M., Koskella, B., Mark Welch, D.B., Martiny, J.B.H., Moran, M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart, F.J., Sullivan, M.B., van Oppen, M.J.H., Weaver, S.C., Webb, E.A., Webster, N.S., 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology 17, 569-586. 10.1038/s41579-019-0222-5

Crawford, J.W., Deacon, L., Grinev, D., Harris, J.A., Ritz, K., Singh, B.K., Young, I., 2012. Microbial diversity affects self-organization of the soil-microbe system with consequences for function. J R Soc Interface 9, 1302-1310. 10.1098/rsif.2011.0679

Crowther, T.W., van den Hoogen, J., Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., Averill, C., Maynard, D.S., 2019. The global soil community and its influence on biogeochemistry. Science 365, eaav0550. 10.1126/science.aav0550

de Graaff, M.-A., Hornslein, N., Throop, H.L., Kardol, P., van Diepen, L.T.A., 2019. Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis, In: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1-44.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K.E., Oliver, A., Ostle, N., Prosser, J.I., Thion, C., Thomson, B., Bardgett, R.D., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nature Communications 9, 3033. 10.1038/s41467-018-05516-7

de Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., Bardgett, R.D., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2, 276-280. 10.1038/nclimate1368

Delgado-Baquerizo, M., Grinyer, J., Reich, P.B., Singh, B.K., 2016. Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. Functional Ecology 30, 1862-1873.

Deveautour, C., Donn, S., Power, S.A., Bennett, A.E., Powell, J.R., 2018. Experimentally altered rainfall regimes and host root traits affect grassland arbuscular mycorrhizal fungal communities. Molecular Ecology 27, 2152-2163.

Di Liberto, T., 2018. A hot, dry summer has led to drought in Europe in 2018, https://www.climate.gov/news-features/event-tracker/hot-dry-summer-has-led-drought-europe-2018.

Droste, N., May, W., Clough, Y., Börjesson, G., Brady, M., Hedlund, K., 2020. Soil carbon insures arable crop production against increasing adverse weather due to climate change. Environmental Research Letters 15, 124034.

Eichorst, S.A., Kuske, C.R., 2012. Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. Appl Environ Microbiol 78, 2316-2327. 10.1128/aem.07313-11

Escalas, A., Hale, L., Voordeckers, J.W., Yang, Y., Firestone, M.K., Alvarez-Cohen, L., Zhou, J., 2019. Microbial functional diversity: From concepts to applications. Ecology and Evolution. 10.1002/ece3.5670

Esperschutz, J., Gattinger, A., Mader, P., Schloter, M., Fliessbach, A., 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiol Ecol 61, 26-37. 10.1111/j.1574-6941.2007.00318.x

Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. Trends in Ecology & Evolution 17, 177-183. https://doi.org/10.1016/S0169-5347(02)02496-5

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15, 579-590. 10.1038/nrmicro.2017.87

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global Consequences of Land Use. Science 309, 570-574. doi:10.1126/science.1111772

García-Palacios, P., Gattinger, A., Bracht-Jørgensen, H., Brussaard, L., Carvalho, F., Castro, H., Clément, J.C., De Deyn, G., d'Hertefeldt, T., Foulquier, A., 2018. Crop traits drive soil carbon sequestration under organic farming. Journal of Applied Ecology 55, 2496-2505.

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mader, P., Stolze, M., Smith, P., Scialabba, N.E.H., Niggli, U., 2012. Enhanced top soil carbon stocks under organic farming. Proceedings of the National Academy of Sciences 109, 18226-18231. 10.1073/pnas.1209429109

Geisen, S., Wall, D.H., van der Putten, W.H., 2019. Challenges and Opportunities for Soil Biodiversity in the Anthropocene. Curr Biol 29, R1036-R1044. 10.1016/j.cub.2019.08.007

Glassman, S.I., Martiny, J.B.H., 2018. Broadscale Ecological Patterns Are Robust to Use of Exact Sequence Variants versus Operational Taxonomic Units. mSphere 3, e00148-00118. 10.1128/mSphere.00148-18

Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS microbiology reviews 37, 112-129.

Hartmann, M., Brunner, I., Hagedorn, F., Bardgett, R.D., Stierli, B., Herzog, C., Chen, X., Zingg, A., Graf-Pannatier, E., Rigling, A., Frey, B., 2017. A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. Molecular Ecology 26, 1190-1206. https://doi.org/10.1111/mec.13995

Hartmann, M., Frey, B., Mayer, J., Mader, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J 9, 1177-1194. 10.1038/ismej.2014.210

He, Z.L., Terry, J.G., Christopher, W.S., Liyou, W., Jost, L., Song, C.C., Zhijian, H., Weimin, W., Baohua, G., Phil, J., Craig, C., Jizhong, 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. The Isme Journal 1, 67-77. doi:10.1038/ismej.2007.2

Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 1998. Ploughing up the wood-wide web? Nature 394.

Henrissat, B., Davies, G., 1997. Structural and sequence-based classification of glycoside hydrolases. Current Opinion in Structural Biology 7, 637-644. https://doi.org/10.1016/S0959-440X(97)80072-3

Hoover, D.L., Rogers, B.M., 2016. Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. Global Change Biology 22, 1809-1820.

Hueso, S., García, C., Hernández, T., 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biology and Biochemistry 50, 167-173.

Iizumi, T., Wagai, R., 2019. Leveraging drought risk reduction for sustainable food, soil and climate via soil organic carbon sequestration. Scientific Reports 9, 19744. 10.1038/s41598-019-55835-y

Islam, W., Noman, A., Naveed, H., Huang, Z., Chen, H.Y.H., 2020. Role of environmental factors in shaping the soil microbiome. Environmental Science and Pollution Research 27, 41225-41247. 10.1007/s11356-020-10471-2

Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology and Biochemistry 40, 2843-2853. https://doi.org/10.1016/j.soilbio.2008.07.030

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza 12, 225-234. 10.1007/s00572-002-0163-z

Jansson, J.K., Hofmockel, K.S., 2020. Soil microbiomes and climate change. Nature Reviews Microbiology 18, 35-46. 10.1038/s41579-019-0265-7

Jin, Z., Shah, T., Zhang, L., Liu, H., Peng, S., Nie, L., 2020. Effect of straw returning on soil organic carbon in rice–wheat rotation system: A review. Food and Energy Security 9, e200. https://doi.org/10.1002/fes3.200

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., Daniel, R., 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Scientific Reports 6, 33696. 10.1038/srep33696

Kardol, P., De Long, J.R., 2018. How anthropogenic shifts in plant community composition alter soil food webs. F1000Research 7, 4-4. 10.12688/f1000research.13008.1

Krause, H.-M., Fliessbach, A., Mayer, J., Mäder, P., 2020. Implementation and management of the DOK long-term system comparison trial, Long-Term Farming Systems Research. Elsevier, pp. 37-51.

Kruger, M., Stockinger, H., Kruger, C., Schussler, A., 2009. DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183, 212-223. 10.1111/j.1469-8137.2009.02835.x

Kundel, D., Meyer, S., Birkhofer, H., Fliessbach, A., Mäder, P., Scheu, S., van Kleunen, M., Birkhofer, K., 2018. Design and Manual to Construct Rainout-Shelters for Climate Change Experiments in Agroecosystems. Frontiers in Environmental Science 6, 1-9. 10.3389/fenvs.2018.00014

Kushwaha, S.K., Manoharan, L., Meerupati, T., Hedlund, K., Ahren, D., 2015. MetCap: a bioinformatics probe design pipeline for large-scale targeted metagenomics. BMC Bioinformatics 16, 65. 10.1186/s12859-015-0501-8

Lal, R., 2013. Intensive Agriculture and the Soil Carbon Pool. Journal of Crop Improvement 27, 735-751. 10.1080/15427528.2013.845053

Lal, R., 2016. Soil health and carbon management. Food and Energy Security 5, 212-222.

Lal, R., Delgado, J.A., Groffman, P.M., Millar, N., Dell, C., Rotz, A., 2011. Management to mitigate and adapt to climate change. Journal of Soil and Water Conservation 66, 276-285. 10.2489/jswc.66.4.276

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31, 814-821. 10.1038/nbt.2676

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

Lessmann, M., Ros, G.H., Young, M.D., de Vries, W., 2021. Global variation in soil carbon sequestration potential through improved cropland management. Global Change Biology 00, 1-16. https://doi.org/10.1111/gcb.15954

Liao, W., Liu, P., Liao, W., Miao, L., 2019. Complete Genome of the Chitin-Degrading Bacterium, Paenibacillus xylanilyticus W4. Genome biology and evolution 11, 3252-3255. 10.1093/gbe/evz241

Lin, X., Feng, Y., Zhang, H., Chen, R., Wang, J., Zhang, J., Chu, H., 2012. Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in North China revealed by 454 pyrosequencing. Environmental science & technology 46, 5764-5771.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjoller, R., Koljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers--a user's guide. New Phytol 199, 288-299. 10.1111/nph.12243

Liu, C., Lu, M., Cui, J., Li, B., Fang, C., 2014. Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis. Global Change Biology 20, 1366-1381.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42, D490-495. 10.1093/nar/gkt1178

Lori, M., Symnaczik, S., Mader, P., De Deyn, G., Gattinger, A., 2017. Organic farming enhances soil microbial abundance and activity-A meta-analysis and meta-regression. PLoS One 12, e0180442. 10.1371/journal.pone.0180442

Madegwa, Y.M., Uchida, Y., 2021. Land use and season drive changes in soil microbial communities and related functions in agricultural soils. Environmental DNA.

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science Vol. 296, 1694-1697.

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., Quero, J.L., Garcia-Gomez, M., Gallardo, A., Ulrich, W., 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Sciences 112, 15684-15689.

Magdoff, F., Weil, R.R., 2004. Soil organic matter in sustainable agriculture. CRC press.

Manoharan, L., Kushwaha, S.K., Ahrén, D., Hedlund, K., 2017a. Agricultural land use determines functional genetic diversity of soil microbial communities. Soil Biology and Biochemistry 115, 423-432. 10.1016/j.soilbio.2017.09.011

Manoharan, L., Kushwaha, S.K., Hedlund, K., Ahren, D., 2015. Captured metagenomics: large-scale targeting of genes based on 'sequence capture' reveals functional diversity in soils. DNA Res 22, 451-460. 10.1093/dnares/dsv026

Manoharan, L., Rosenstock, N.P., Williams, A., Hedlund, K., 2017b. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. Applied Soil Ecology 115, 53-59. 10.1016/j.apsoil.2017.03.012

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

Maron, P.A., Mougel, C., Ranjard, L., 2011. Soil microbial diversity: Methodological strategy, spatial overview and functional interest. C R Biol 334, 403-411. 10.1016/j.crvi.2010.12.003

Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. The Isme Journal 7, 830-838. 10.1038/ismej.2012.160

Marulanda, A., Azcon, R., Ruiz-Lozano, J.M., 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by Lactuca sativa plants under drought stress. Physiologia Plantarum 119, 526-533.

Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.), 2021. IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. In Press.

McLaughlin, A., Mineau, P., 1995. The impact of agricultural practices on biodiversity. Agriculture, Ecosystems and Environment 55, 201-212.

Meisner, A., Bååth, E., Rousk, J., 2013. Microbial growth responses upon rewetting soil dried for four days or one year. Soil Biology and Biochemistry 66, 188-192.

Meisner, A., Jacquiod, S., Snoek, B.L., ten Hooven, F.C., van der Putten, W.H., 2018. Drought Legacy Effects on the Composition of Soil Fungal and Prokaryote Communities. Frontiers in Microbiology 9. 10.3389/fmicb.2018.00294

Mettel, C., Kim, Y., Shrestha, P.M., Liesack, W., 2010. Extraction of mRNA from soil. Applied and Environmental Microbiology 76, 5995-6000. 10.1128/AEM.03047-09

Mewis, K., Lenfant, N., Lombard, V., Henrissat, B., 2016. Dividing the large glycoside hydrolase family 43 into subfamilies: a motivation for detailed enzyme characterization. Applied and Environmental Microbiology 82, 1686-1692.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., 2017. Soil carbon 4 per mille. Geoderma 292, 59-86.

Mohammadipanah, F., Wink, J., 2016. Actinobacteria from Arid and Desert Habitats: Diversity and Biological Activity. Frontiers in Microbiology 6. 10.3389/fmicb.2015.01541

Nacke, H., Fischer, C., Thürmer, A., Meinicke, P., Daniel, R., 2014. Land Use Type Significantly Affects Microbial Gene Transcription in Soil. Microbial Ecology 67, 919-930. 10.1007/s00248-014-0377-6

Nielsen, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. European Journal of Soil Science 62, 105-116. 10.1111/j.1365-2389.2010.01314.x

Nilsson, R.H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., Tedersoo, L., 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17, 95-109. 10.1038/s41579-018-0116-y

Ochoa-Hueso, R., Collins, S.L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W.T., Sinsabaugh, R.L., Smith, M.D., Knapp, A.K., Power, S.A., 2018. Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Glob Chang Biol 24, 2818-2827. 10.1111/gcb.14113

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M.G.A., Sieverding, E., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology and Biochemistry 42, 724-738. 10.1016/j.soilbio.2010.01.006

Orgiazzi, A., Bardgett, R.D., Barrios, E., 2016. Global soil biodiversity atlas. European Commission.

Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology 8, 523-529. 10.1038/nrmicro2367

Popovic, A., Parkinson, J., 2018. Characterization of eukaryotic microbiome using 18S amplicon sequencing, Microbiome Analysis. Springer, pp. 29-48.

Poretsky, R.S., Bano, N., Buchan, A., LeCleir, G., Kleikemper, J., Pickering, M., Pate, W.M., Moran, M.A., Hollibaugh, J.T., 2005. Analysis of microbial gene transcripts in environmental samples. Appl Environ Microbiol 71, 4121-4126. 10.1128/aem.71.7.4121-4126.2005

Powlson, D.S., Riche, A.B., Coleman, K., Glendining, M.J., Whitmore, A.P., 2008. Carbon sequestration in European soils through straw incorporation: Limitations and alternatives. Waste Management 28, 741-746. https://doi.org/10.1016/j.wasman.2007.09.024

Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biology and Biochemistry 131, 28-39.

Prosser, I.J., 2002. Molecular and functional diversity in soil micro-organisms. Plant and Soil 244, 9-17.

Prosser, J.I., 2015. Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. Nat Rev Microbiol 13, 439-446. 10.1038/nrmicro3468

Rawlings, N.D., Barrett, A.J., Bateman, A., 2012. MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res 40, D343-350. 10.1093/nar/gkr987

Rawls, W.J., Pachepsky, Y.A., Ritchie, J.C., Sobecki, T.M., Bloodworth, H., 2003. Effect of soil organic carbon on soil water retention. Geoderma 116, 61-76. https://doi.org/10.1016/S0016-7061(03)00094-6

Rengel, Z., 2007. The role of crop residues in improving soil fertility, Nutrient cycling in terrestrial ecosystems. Springer, pp. 183-214.

Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiology Ecology 78, 17-30. 10.1111/j.1574-6941.2011.01106.x

Ruiz-Lozano, J.M., 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13, 309-317.

Rundlöf, M., Smith, H.G., Birkhofer, K., 2016. Effects of organic farming on biodiversity. eLS, 1-7.

Sanders, I.R., 2004. Intraspecific genetic variation in arbuscular mycorrhizal fungi and its consequences for molecular biology, ecology, and development of inoculum. Canadian Journal of Botany 82, 1057-1062. 10.1139/b04-094

Scheurwater, E., Reid, C.W., Clarke, A.J., 2008. Lytic transglycosylases: Bacterial space-making autolysins. The International Journal of Biochemistry & Cell Biology 40, 586-591. https://doi.org/10.1016/j.biocel.2007.03.018

Schimel, J.P., 2018. Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. Annual Review of Ecology, Evolution, and Systematics 49, 409-432. 10.1146/annurev-ecolsys-110617-062614

Schlaeppi, K., Bender, S.F., Mascher, F., Russo, G., Patrignani, A., Camenzind, T., Hempel, S., Rillig, M.C., van der Heijden, M.G.A., 2016. High-resolution community profiling of arbuscular mycorrhizal fungi. New Phytol 212, 780-791. 10.1111/nph.14070

Schmid, J., Heider, D., Wendel, N.J., Sperl, N., Sieber, V., 2016. Bacterial Glycosyltransferases: Challenges and Opportunities of a Highly Diverse Enzyme Class Toward Tailoring Natural Products. Frontiers in Microbiology 7. 10.3389/fmicb.2016.00182

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56. 10.1038/nature10386

Schnoor, T.K., Lekberg, Y., Rosendahl, S., Olsson, P.A., 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. Mycorrhiza 21, 211-220. 10.1007/s00572-010-0325-3

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Consortium, F.B., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109, 6241-6246. 10.1073/pnas.1117018109

Schüβler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research 105, 1413-1421. 10.1017/s0953756201005196

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229-232.

Sharma, S.B., Gobi, T.A., 2016. Impact of Drought on Soil and Microbial Diversity in Different Agroecosystems of the Semiarid Zones, In: Hakeem, K.R., Akhtar, M.S., Abdullah, S.N.A. (Eds.), Plant, Soil and Microbes: Volume 1: Implications in Crop Science. Springer International Publishing, Cham, pp. 149-162.

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic stoichiometry and ecological theory. Annual Review of Ecology, Evolution, and Systematics 43, 313-343.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11, 1252-1264. 10.1111/j.1461-0248.2008.01245.x

Smith, S.E., Read, D.J., 2010. Mycorrhizal symbiosis. Academic press.

Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. The Isme Journal 3, 992-1000. 10.1038/ismej.2009.43

Spinoni, J., Vogt, J.V., Naumann, G., Barbosa, P., Dosio, A., 2018. Will drought events become more frequent and severe in Europe? International Journal of Climatology 38, 1718-1736.

Stam, M.R., Danchin, E.G., Rancurel, C., Coutinho, P.M., Henrissat, B., 2006. Dividing the large glycoside hydrolase family 13 into subfamilies: towards improved functional annotations of α-amylase-related proteins. Protein Engineering, Design and Selection 19, 555-562.

Stockinger, H., Kruger, M., Schussler, A., 2010. DNA barcoding of arbuscular mycorrhizal fungi. New Phytol 187, 461-474. 10.1111/j.1469-8137.2010.03262.x

Tedersoo, L., Tooming-Klunderud, A., Anslan, S., 2018. PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. New Phytol 217, 1370-1385. 10.1111/nph.14776

Thomson, B.C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R.I., Hannula, S.E., Buée, M., Mougel, C., Ranjard, L., Van Veen, J.A., Martin, F., Bailey, M.J., Lemanceau, P., 2015. Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. Soil Biology and Biochemistry 88, 403-413. https://doi.org/10.1016/j.soilbio.2015.06.012

Torsvik, V., Øvreås, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5, 240-245.

Tsiafouli, M.A., Thebault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jorgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setala, H., Tzanopoulos, J., Uteseny, K., Pizl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Glob Chang Biol 21, 973-985. 10.1111/gcb.12752

Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3, e2527. 10.1371/journal.pone.0002527

van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11, 296-310. 10.1111/j.1461-0248.2007.01139.x

Verbruggen, E., Roling, W.F., Gamper, H.A., Kowalchuk, G.A., Verhoef, H.A., van der Heijden, M.G.A., 2010. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytol 186, 968-979. 10.1111/j.1469-8137.2010.03230.x

Vogel, E., Donat, M.G., Alexander, L.V., Meinshausen, M., Ray, D.K., Karoly, D., Meinshausen, N., Frieler, K., 2019. The effects of climate extremes on global agricultural yields. Environmental Research Letters 14, 054010.

Wall, D.H., Bardgett, R., Behan-Pelletier, V., Herrick, J., Jones, T., Ritz, K., Six, J., Strong, D., van der Putten, W.H., 2012. Soil Ecology and Ecosystem Services. Oxford University Press. 10.1093/acprof:oso/9780199575923.001.0001

Wallenstein, M.D., Burns, R.G., 2011. Ecology of Extracellular Enzyme Activities and Organic Matter Degradation in Soil: A Complex Community-Driven Process, Methods of Soil Enzymology, pp. 35-55.

Xiang, D., Verbruggen, E., Hu, Y., Veresoglou, S.D., Rillig, M.C., Zhou, W., Xu, T., Li, H., Hao, Z., Chen, Y., Chen, B., 2014. Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China. New Phytol 204, 968-978. 10.1111/nph.12961

Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P.G., Schmidt, T.M., Zhou, J., 2013. Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. Appl Environ Microbiol 79, 1284-1292. 10.1128/AEM.03393-12

Yang, C., Hamel, C., Schellenberg, M.P., Perez, J.C., Berbara, R.L., 2010. Diversity and functionality of arbuscular mycorrhizal fungi in three plant communities in semiarid Grasslands National Park, Canada. Microb Ecol 59, 724-733. 10.1007/s00248-009-9629-2

Yang, T., Lupwayi, N., Marc, S.-A., Siddique, K.H.M., Bainard, L.D., 2021. Anthropogenic drivers of soil microbial communities and impacts on soil biological functions in agroecosystems. Global Ecology and Conservation 27, e01521. https://doi.org/10.1016/j.gecco.2021.e01521

Yousaf, M., Li, J., Lu, J., Ren, T., Cong, R., Fahad, S., Li, X., 2017. Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. Scientific Reports 7, 1270. 10.1038/s41598-017-01412-0

Zhang, J., Wang, F., Che, R., Wang, P., Liu, H., Ji, B., Cui, X., 2016. Precipitation shapes communities of arbuscular mycorrhizal fungi in Tibetan alpine steppe. Sci Rep 6, 23488. 10.1038/srep23488

Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nature Communications 11, 1-10.

Zomer, R.J., Bossio, D.A., Sommer, R., Verchot, L.V., 2017. Global Sequestration Potential of Increased Organic Carbon in Cropland Soils. Scientific Reports 7, 15554. 10.1038/s41598-017-15794-8

Acknowledgments

Doing a PhD was not a sprint, it was a marathon! It would have never been possible to reach the finish line without the people I met on the way. I feel very grateful to be surrounded by people how gave me support, motivation, offered understanding, challenged me and brought a smile on my face during the past few years.

Katarina. Having you as a supervisor has been a pleasure. Working under your supervision has been very enjoyable and I have learned a lot. Thanks for giving me the opportunity to grow and work in such a nice environment. You have been available whenever I needed your feedback and support. Your ability to see the progress that I have made when I felt stuck and your ability to put everything into perspective, have been invaluable for me. Moreover, I am happy we share the same enthusiasm for nature and winter activities, especially skiing. You gave me many advice to explore Sweden and I am grateful you have always been very understandable when I needed a break in nature to recharge my batteries. Thank you for everything!

Dag. When I started my PhD, I did not know anything about bioinformatics, not even what the server and terminal are or how to write a script. You gave me the best possible introduction into this exciting field and helped me with my beginner coding struggles. I really appreciate all the support and advice you gave me. Thank you!

Pål Axel. Thank you for your support during these years! Thanks for all discussions on my first project about arbuscular mycorrhizal fungi.

Allan. Thanks for being my examiner through this thesis. Our meetings have been very interesting, and your comments on my work and progress were always constructive. Sometimes you were a bit sceptical about my ambitious plans for the upcoming projects, which gave me even more motivation. You also taught me to be realistic with my goals.

Lokesh. I cannot express how grateful I am you became a part of this journey! You were always available to offer help, give suggestions, being patient in explaining bioinformatics I did not understand. I enjoyed our scientific and non-scientific discussions very much. Thanks for cheering me up in the last months of this journey, everything seemed much easier. Thank you for everything!

Helene. Thank you for the positive energy and the smile I received every single morning. You always cared about how I feel and you always found the right words

to cheer me up. Thanks for organizing the fieldwork, you thought of every single detail and I cannot imagine how that would go without you. Thank you!

John. Thank you for being my bioinformatics advisor for the last two years. Our meetings have always been very interesting, I enjoyed them very much and learned a lot from you! You have always been very enthusiastic about my research, and it was nice to receive feedback and insightful perspectives about my research from someone outside the soil microbial ecology field. Thanks for all the discussions and inputs!

Karolina. The mentoring program was one of the best experiences I had during my PhD. I had the chance to talk with you about my future, life in general and even more. Thanks for all the fikas, walks, advice, kind and supportive words. I am very happy you were part of this journey. Thank you!

Sandeep. Thank you for helping me in dealing with bioinformatics problems, always with a positive attitude and a big smile on your face.

María. It was a pleasure to share the office with you for the first two years. You gave me many useful advice, tried to teach me Swedish, always listened to me or give your opinion when I was in doubt. I also enjoyed doing fieldwork with you, we had a great time!

Mats Hansson. We did not meet many times, but I always knew if something goes wrong or I need advice, your doors are open.

Thanks to everyone from the **SoilClim consortium**. We were very busy with the fieldwork across Europe and scientific discussions, but luckily, we also had a chance to have fun and enjoy ourselves.

Peter Olsson. Thank you for helping me with the statistics, always taking time to explain in detail how models work and making sure I understood it. You have been an amazing statistical teacher to me. Thank you for that!

Mara. You brought so much joy to my life! Just seeing you always made me happy and brought a smile to my face. Drinking morning tea, going for a lunch run, spinning, chatting or laughing. These are only some of the happy moments I missed very much in the past two years. I cannot wait to see you soon!

Ainara. It was nice to have you around in good and bad days. You are such an inspiration just being you. Thanks for all the dinners, walks and conversations we had together. Thanks for being such a great friend!

Raphael, Martin and **Carlos.** Thank you for all the great moments we spent together! Dinners, BBQ, midsummers… With you guys was always a lot of fun and you brought a lot of good and positive energy to my life.

Dimitri. You always welcomed me in your office when I needed a conversation. Thanks for listening to all my scientific-related problems and giving advice and

input on my work. I am very grateful you shared your knowledge and own experience with me. Thank you!

Johannes. Thank you for giving me the chance to share the lab meetings with your group. It was a very nice experience and helped me to obtain a broader perspective about the soil microbial ecology field.

Daniel, Lettice, Carla, Mingyue, Sara, Albert, Margarida and other past members of the **Rousk lab.** Thanks for the weekly lab meetings, delicious breakfasts and cakes, discussions, the knowledge you shared with me and all the fun moments.

Aivars. We shared many ups- and downs during these years. Thanks for all the extended lunch breaks, the moments of chatting, laughing and complaining.

Thanks to **GENECO** for organizing the workshops, courses and meetings. It was a great opportunity for me to learn new things and exchange ideas with many great people.

GENECO mentees. Thanks for your understanding, support and meaningful conversations.

Thanks to every single person at the **Biodiversity unit**, for our Monday breakfasts, winter meetings, interactions, and discussions throughout the years. It was a great pleasure to work in such a positive environment.

Thanks to past and present PhD students at the Biodiversity unit: **Elsa, Leidys, Hamid, Maria, Chon, Dafne, Carsten, Johanna, Hampus, Cecilia** and **Josefin** for the company, fikas, and lunches.

Thanks to all the people I met during these years either at the Friday pub, BLAM, football, or badminton. **Romain, Pierre, Pablo, David, Linus, Ann-Kathrin, Franca, Saeed, Sandra, Lulu, Qinyang, Violeta, Fabian, Micaela, Juan Pablo, Oscar, Alex, Milda, Humberto, Katarina** and **Jesbol.** Thanks for all the moments that we have shared together!

Katarina. It was quite a dream that we ended up in the same city after Innsbruck. Even if only for a short while. You introduced me to En Svensk Klassiker and many more Swedish traditions. It was always nice to spend time together in nature and exploring new places. Thanks to Patrik and your families to make me feel welcome and at home every time I visited you.

Rebeka, David and **Ana.** Slovenian crew in Lund! Thanks for all the great moments we had together! Dinner, celebrations, hikes, fikas, cooking and baking traditional Slovenian dishes. It has been an amazing time and I am very happy we met each other!

Marcella. We had so many great walks, talks, spinning sessions and fikas together. I enjoyed every single minute we spend together! Thank you for that!

Jolanta. You were one of the first people I get to know when I moved to Sweden. Thank you for all the walks, fikas and dinners!

Michail. My cycling buddy! Thanks for all the cycling trips! We had a lot of fun exploring the hidden gems of Skåne, which I will never forget. Thanks for this!

Justine. We have not seen each other much in recent years, but our yearly trips to the mountains, for hiking, cycling, running have always been the best! Thank you for being such a great friend. I am already looking forward to our next trip to the mountains!

My friends from back home!

Tjaša. Thank you for being such a supportive and great friend! I feel so grateful to have you in my life. You have always been there for me, in good and bad days. You are an amazing person and I am looking forward to many new adventures together!

Tajda and **Anja.** I was always looking forward to our sports adventures; cycling, cross country skiing, running and hiking! I am very grateful for our friendship and cannot wait for more adventurous moments in the future!

Sara, Erika, Žiga and **Janže.** It was always nice to hang out and chat with you. I am very much looking forward to seeing you soon.

Anja, Nina and **Tjaša.** I am super happy we reconnected after many years. I cannot wait for more gossiping and gin & tonics in the near future!

Matic. Thanks for always being up for an adventure, either for a road trip to Asia, hiking in Sweden or an easy walk to the neighbouring hill!

Ema. Thank you for painting this beautiful cover for me!

My family. My sister, mom and dad. My biggest supporters, thank you for everything!