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Identifying microbial indicators of land-use change and determining their relevance for soil ecosystem services

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Declaration of Authorship

I, Melanie ARMBRUSTER, declare that this thesis titled, "Identifying microbial indicators of land-use change and determining their relevance for soil ecosystem services" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
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Date: 01.09.2021

"We are free to change the world and start something new in it."

Hannah Arendt (1972)

Abstract

Identifying microbial indicators of land-use change and determining their relevance for soil ecosystem services

Microbial communities drive many soil ecosystem services and are heavily influenced by land use change and intensive agriculture. In order to manage land sustainably, there is a need to close critical knowledge gaps as to how management affects multiple soil services and their resilience to future change, and the functional importance of microbial communities in this complex system. While local studies revealed responses of biodiversity to land use, there is a need to study the landscape scale and synthesise findings in order to predict different soil, land use and climatic contexts. In this thesis, microbial indicators were determined for contrasting land use types and their relation with organic matter recovery tested. At the large scale, this relationship was confirmed in a restoration chronosequence of calcareous grasslands, which was limited to high pH soils and revealed consistent responses of distinct bacterial and fungal taxa (verrucomicrobial DA101 and *Ca. Xiphinematobacter* and *alpha*-Proteobacteria in low intensity, vs. ammonia-oxidising archaea and bacteria in cropland). These indicator taxa were confirmed in a mesocosm experiment, in which contrasting soil communities were transferred to a degraded long term bare fallow soil and their relative contribution to C related soil functions assessed across a pH gradient. While pH significantly changed extracellular enzyme activities, respiration and OM contents, all tested soil functions were independent of land use of the applied bacterial community, pointing us to a non-linear relationship between bacterial diversity and ecosystem functionality. In contrast, a survey of Conservation Agriculture field experiments which covered reduced tillage, cover cropping and manure application in 14 different farm systems, did not confirm the indicative meaning of these organisms and the effect of Site was stronger than any management change. Nevertheless, I found distinct communities depending on management intensity, with tillage having stronger implications than cover

cropping or the addition of organic amendments. In synthesis, soil microbial communities were mainly driven by pH and site-dependent factors, but there were consistent indicators of recovered organic matter stocks and C related functions in high pH systems. Farming intensity simultaneously affected soil C contents and prokaryote and eukaryote communities, with an associated change in soil pH. Restoration of degraded soil functions which are related to carbon cycling via application or manipulation of distinct microbial communities is thus clearly pH-dependent and has to consider individual site-related factors. Further investigations considering other soil (pH) systems and managements are hence needed to confirm specific indicators and their relative contribution to soil health. With the help of modern soil functional assays as well as new isotopic, metagenomic and transcriptomic tools, new insights into the functional capacity of soils and their microbiomes will shed light on their resistance-resilience to land use and climate change.

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List of Abbreviations

16S rRNA	bacterial ribosomal RNA marker gene
AMF	Arbuscular Mycorrhiza Fungi
AOA/AOB	Ammonia Oxidising Archaea/ Bacteria
ASV	exact Amplicon Sequence Variance
CA	Conservation Agriculture
CC	Cover Crop(s)
ESS	Eco System Service (s)
FYM	Farm Yard Manure
ITS	Internal transcribed spacer
LOI	Loss-on-Ignition
NGS	next generation sequencing
OC	Organic Carbon
OM	Organic Matter
OTU	Operational Taxonomic Unit
SOC	Soil Organic Carbon
SOM	Soil Organic Matter

Chapter 1

General Introduction

Soil provides a fundamental media for the growth of plants and animals which provide the food to support human populations. In addition, soil provides a space for above and below ground processes which affect global nutrient cycles. For example, soil acts as a sink or source of carbon and nitrogen, and such processes also contribute to greenhouse gas emissions, which can impact on the global climate (Smith et al., 2016). The high biological diversity found in soils interacts with plant inputs and other factors to catalyse these processes; yet there are large uncertainties over both the extent of soil biodiversity, its controlling drivers, and its importance in regulating soil processes (Nielsen et al., 2011; Nannipieri et al., 2003).

This is particularly true for microorganisms, which are key players in soil food webs, though the vast majority of representatives remain to be characterised and described. It is widely accepted, that land-use change, in particular conversion of natural habitats into croplands damages soils, their functions and the diversity they host, but that a growing human population will need increased amounts of food and larger areas of domesticated land in the next decades. New research is therefore needed to fully understand both the effects of land management change on soil biodiversity, as well as the resultant impacts on soil functionality.

This thesis presents both observational research examining the impacts of land-use change on soil microbial biodiversity and functionality, as well as experimental approaches to decipher microbial links to specific soil functions. A key focus is in the use of new molecular technologies to assess microbial biodiversity responses, which prior to the advent of molecular biology was typically difficult to asses. The

research was conducted both to address fundamental issues in contemporary microbial ecology, in that it seeks to address the ecological drivers of changes in diverse and enigmatic soil microbial communities as well as establish functional relevance.

It also addresses broader political and industry relevant issues pertaining to sustainable approaches to land management and soil health, and identifying biotic indicators responsive to land-use change.

Before introducing the specific aims, I will present a review beginning with the broader political context of how natural environments and soils are now considered a valuable resource alongside primary productivity to form a policy agenda to enable sustainable land management (McCarthy et al., 2012; Daily, 1997). This will be followed by a synthesis of knowledge on soil biodiversity incorporating methodologies and state of knowledge of land-uses effects.

Finally, I will end on identifying key knowledge gaps and conclude with an outline of the thesis aims.

1.1 Ecosystem Services and Nature's contributions to People

Soils have received considerable recent attention from both political and scientific research perspectives, in large part due to global efforts to include nature or "natural capital" into human economic frameworks. The term Ecosystem Service (ESS) is defined as "a flow generated by the ecosystem including ecological interactions and information which are useful to human beings." and was principally introduced in the Millenium Ecosystem Assessment (Reid and Al., 2005).

In addition, new definitions evolved around the term and frameworks expanded their perspectives so that, depending on the context, "ecosystem services sometimes require human input" (La Notte et al., 2017a). The principal aim of the Millennium Ecosystem Assessment and indeed the formulation of ESS concepts was to highlight that whilst nature does not have immediately obvious monetary value, it provides human society with numerous benefits and currently overlooked in current economic activities. This report defined four categories of ESS, that support human life and well-being:

- Provisioning Services: accounting for the production of food, wood, fibre and clean water,
- Supporting (not directly used by humans): nutrient cycling, soil formation, primary biomass production, habitat provision,
- Regulating: climate regulation, protection from drought or flood, water storage and purification,
- Cultural: spiritual, aesthetic, educational, recreational values.

Following the Millenium Ecosystem Assessment in 2015, the 17 Sustainable Development Goals were launched by the United Nations General Assembly, which aim at global human and planetary well being and require thus well functioning ecosystems.

Originally, the ecosystem services concept was thought to protect nature, by underlining the priceless value of Nature's substantial contributions to humanity on the one hand, and on the other hand by economic quantification of ecological processes, aiming at the provision of policy relevant decision support (McCarthy et al., 2012; Daily, 1997). Goods and services provided by natural and agricultural ecosystems are priceless and their damage remains often irreversible. The financial value of all ESS provided by global biomes sums up to an average of US $ 33 trillion per year (Costanza et al., 1997). The cost to protect, conserve and maintain the benefits associated with global biodiversity for example, were estimated to $ 76 billion per year, but unfortunately, the amount of money actually invested in nature conservation is magnitudes below that (McCarthy et al., 2012). In many cases, human activity causes habitat fragmentation, climate change, land-use change and pollution, which disrupt the provision of ESS (Foley et al., 2005; Tilman et al., 2002; Tsiafouli et al., 2015).

To mitigate these negative anthropogenic effects on global and local ecosystems, reliable monitoring programmes and conservation efforts are required, that take into account all dynamic fluxes and complex feed backs of functional significance.

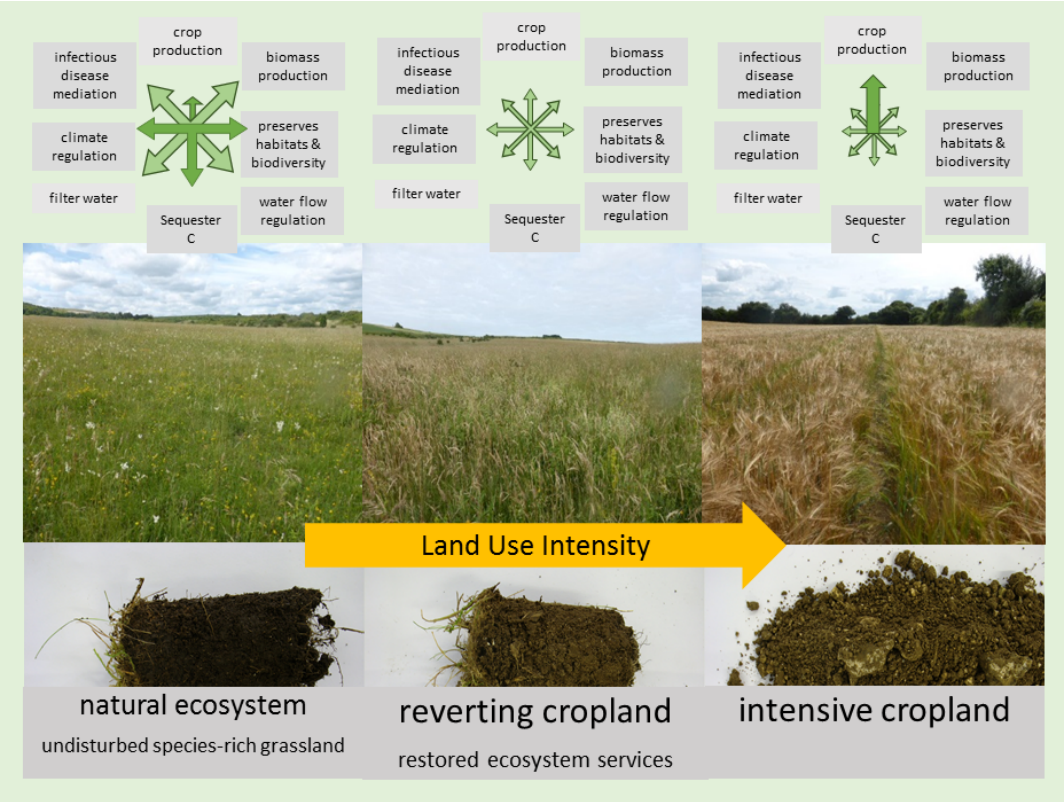

FIGURE 1.1: **Soil ecosystem services provided by natural and agricultural ecosystems.** Trade-offs of ecosystem services provided by natural, undisturbed habitats (left panel, calcareous grassland), intensively managed cropland (right panel, monoculture of cereals, regular plough/fertiliser/pesticide application), and 25 years of grassland natural regeneration in South England. Each soil core corresponds to the adjacent landscape photography. Textual information adapted from Foley et al., 2005, photographs of soils taken by myself as part of the survey presented in Chapter 2

1.1.1 Soil as a resource - what ecosystem services does it provide?

Many essential ecosystem functions are provided by soils, without which human life on earth would not be possible. They range from the provision of food, fibre and fuel, water purification and reduction/detoxification of harmful contaminants, to regulating services like climate regulation, flood prevention and nutrient cycling. Moreover, soils provide habitat for a great variety of organisms, and as such store a largely untapped genetic resource for potential biotechnological exploitation (Figure 1.1). Even human health is strongly influenced by soil health, as soil biodiversity has even been observed to influence the human gut microbiome, which is essential to our well-being (Walsh et al., 2019). Furthermore, cultural services are provided

by soils, as they host archaeological, spiritual and historical treasures and resources of past human civilisations on the one hand and provide building ground and construction materials for modern civil engineering, as well as building the ground layer for valuable aesthetic landscapes on the other side (FAO et al., 2019).

Given the increased recognition of the multitude and diversity of essential services provided by soils, terms such as "soil health" or "soil quality" are increasingly being used to reference aspirational targets for a variety of applications, be it landscape restoration, new crop management or other policy agendas. It is noteworthy, that the use of such terms pre-dates the concepts of ESS, with use of such terms dating to the early 20th century (Wallace, 1910). However, the exact meaning of these terms has often been ill-defined which is likely a consequence of the multitude of potential uses for soil.

The new ESS frameworks therefore may offer some help, in that they implicitly recognise multiple service delivery and the existence of trade-offs. Importantly, the term 'soil health' describes rather the functional capacity of service delivery than the actually performed ESS, hence it is not recommended to focus solely on the output of ESS in soil health assessments (Kibblewhite, Ritz, and Swift, 2007; Karlen et al., 2019).

Despite the ongoing debate over definitions, there have been several large scale attempts at quantifying soil health, by focusing on specific functions or services. The European Commission's Joint Research Centre recently estimated that 60 -70 % of EU soils are at risk of physical degradation and classified as "unhealthy" (ANNEX 1, Soil Health and Food Mission Board and the European Commission's Joint Research Centre Review 2020).

A global loss of fertile soils due to intensive agriculture is estimated to reach 24 billion tonnes every year, while 970 million tonnes of European soils get lost to erosion (Pierzynski et al., 2017) (http://www.fao.org/soils-portal/). Globally, soils are threatened by deforestation, population growth, urban expansion, pollution, climate and unsustainable land-use (change) (Smith et al., 2016).

These drivers of degradation cause erosion, acidification, pollution, sealing, compaction, nutrient imbalance, salinization, a depletion of soil organic matter contents,

and species loss and reduced biological diversity (FAO and ITPS, 2015). European biodiversity is massively threatened due to intensive agricultural management and the related soil degradation (Biodiversity and Ecosystem Services, 2018). During the last century, application rates of fertilisers and pesticides increased constantly, with crop and cereal production following this trend. Simultaneously, nitrogen inputs from legumes and plant growth stimulants as well as phosphorus mobilisation in terrestrial and aquatic ecosystems exceeded natural limits. This is partly due to the low nutrient use efficiency of plants in conventional agricultural systems. While 30 - 50 % of N fertiliser and about 45 % P fertiliser is taken up by crops, most of the nutrients get lost to run off from fields (Tilman et al., 2002), polluting groundwater and surface waters which can reach toxic levels for human consumption.

It is therefore clear that intensive agriculture to feed growing populations, is harming the ability of soils to deliver vital services to society. Inability to halt this decline in soil health could lead to a number of direct and indirect consequences for future human societal well being. Without mitigation approaches or technological adaptation, humans may suffer from an insecure supply of food, nutrition and clean water, which could lead to wider social conflicts, poverty and migration issues. These issues may be further confounded, by changes in the global climate, of which again soils can regulate through acting as a source and sink of greenhouse gases depending on land-use type and intensity (Smith et al., 2016; FAO et al., 2019; FAO and ITPS, 2015).

Globally, soils release the second biggest carbon flux with estimates up to 98 Pg C annually, which is a magnitude higher than anthropogenic C emissions from fossil fuel combustion (Raich and Schlesinger, 1992; Bond-Lamberty and Thomson, 2010). An important recent document defined nine planetary boundaries, which may not be transgressed to allow human development on planet Earth, several of which are highly pertinent to soil systems. Three of nine interlinked planetary boundaries, namely climate change, biodiversity loss and the nitrogen cycle, have already been overstepped in 2009 (Rockström et al., 2009). Another planetary boundary, land-use change, is still in the safe operating space, but it remains the main cause of species extinction (Sala et al., 2000). Furthermore, land conversion, especially in South Asia

and South America, contributes to global climate change through large release of greenhouse gases (GHGs). With 47 % of methane and 58 % of N_2O, agricultural land-use is a major driver of GHG emissions world wide, additionally to the immense release of carbon dioxide (Godfray et al., 2011).

Clearly for the benefit of future generations, society needs to develop new approaches for feeding global populations which minimise harmful impacts to soil ESS. The current aim for increased food production to feed a growing population, sounds alarming, especially where an increased demand of up to 70 % is expected (Lal, 2019). In fact, the food produced today is enough to feed 1.2 billion people, but under- and malnutrition are globally on the rise. Additionally, due to climatic changes, there will be up to 50 % less agricultural productivity in certain areas of the world by 2050 (Institut fur Welternaehrung, Wilfried Bommert 2019). Complex systems in Conservation Agriculture, which are in unison with natural ecosystems, as well as restoration measures on degraded land are therefore necessary to achieve global sustainability (Lal, 2019).

Sustainable agricultural management

To reduce the negative impacts of intensive farming soil ecosystems, a more sustainable approach to land-use will be required and many new options are currently being considered. Globally, there are aspirational aims to reduce food waste along the whole production-supply-consumer chain and maintaining less wasteful diets (Westhoek et al., 2014; Erisman et al., 2008). This would optimally require a shift from industrial meat to a pulses based diet with eventually smaller portions, which may also benefit human health in the countries suffering from malnutrition or overnutrition in wealthy, industrialised countries (Sutton et al., 2011; Westhoek et al., 2014; Lal, 2019). However, such efforts would require a large change in human dietary preferences which may be difficult to implement (Westhoek et al., 2014).

At the state level, policies could be adopted to optimise use of natural resources to deliver multiple ecosystem benefits, the so called "land sparing" approach. Here it is thought that it may be better to protect undisturbed sites like carbon rich upland soils and intensify agriculture at denoted areas already in arable use (Pretty

et al., 2018; Godfray et al., 2011; Lal, 2019). For example, agricultural intensification in under-yielding areas provides the opportunity to increase yields on existing cropland without over-fertilisation. It has been documented that small scale farm practice in Zimbabwe uses only 13 % of the usual nitrogen input of high income, economically stronger countries (Tilman et al., 2011). A moderate or even minimal application of N fertilizer in combination with technology and knowledge transfer in such areas can increase grain yields by up to 40 % and return their financial investment by up to 400 % (Twomlow et al., 2010).

Environmental impacts of such sustainable intensification would be considerably lower than land-clearing or change from natural forest or grasslands to agricultural land-use, avoiding a loss of soil C, species diversity and adverse greenhouse gas emissions (Tilman et al., 2011; Putte et al., 2010; Poeplau and Don, 2015). However, such approaches may only be relevant for certain developing countries, given that in developed countries such as the UK, much land is already under highly intensive agricultural management and further expansion of agricultural land is not a counter option. It is likely, that for Western developed countries efforts need to focus on conserving and restoring natural habitats, whilst enhancing production with approaches which minimise degradation of soil ecosystem services - the so called "conservation agriculture" approaches (Putte et al., 2010; Poeplau and Don, 2015).

Conservation agriculture and organic farming practices are receiving considerable uptake in Western societies as both farmers and consumers become more aware of the need to preserve soil health, and the potentially negative impacts of intensively produced food to both human well being and the environment. Though these approaches are diverse, they encompass three main principles: 1) a permanent soil cover by plants; 2) minimum soil disturbance and reduction of inorganic inputs; and 3) crop diversification and stimulation of biota (Lal, 2019). They make use of crop rotations, cover crops, reduced tillage, agroecology, vegetated field margins (hedges, trees) and application of organic amendments like compost or farm yard manure. Though often touted as "new","alternative" or "eco" management, these approaches are largely based upon old farming practices widely adopted prior to the post war

period of western agricultural intensification. Modern technology, in the form of advanced farm machinery such as seed drills and targeted fertiliser application technology now enables the use of such approaches whilst maintaining the intensive cultivation required to feed large numbers of people. Such eco-intensification approaches are believed to restore soil health by reducing synthetic inputs and enhancing nutrient use efficiency, and the adoption of a "system-based" conservation agriculture approach is widely thought to be able to maintain agricultural productivity in the long term whilst minimising deleterious environmental consequences (Poeplau and Don, 2015; Putte et al., 2010; Lal, 2019; Pretty et al., 2018).

Despite the widespread promotion and adoption of such approaches, there exists large uncertainties over the claimed benefits- whether for the farmer, consumer or indeed the environment. Consequently there is little in the way of current government policy to incentivise implementation. Whilst there has been much recent talk of recognising and rewarding farmers and land owners as managers of the most valuable of all terrestrial resources, this has yet to translate into formal policy (Tilman et al., 2011; Tilman et al., 2002). Soil organic carbon is a widely used soil quality indicator (Bongiorno et al., 2019) and has been the focus of much of the evidence base in evaluating new management efficacy, because of its fundamental role in processes related to nutrient cycling and soil health. As most terrestrial carbon is stored in soils, soil carbon management should be considered a high priority in agriculture, to maintain ecosystem multi-functionality in the long term. New proposed initiatives such a 4 per mil (Minasny et al., 2017) argue that implementation of sustainable crop management could offset anthropogenic carbon emissions by capturing and storing carbon in soil.

However, there has been much critique as to whether approaches such as minimum tillage actually result in net carbon accrual, or whether they simply cause carbon redistribution within the soil profile (Powlson et al., 2012; Powlson et al., 2014). As a result of these concerns, focus once again has turned to preserving and restoring habitats which naturally support higher carbon, such as peats (Smith et al., 2016). Wider uncertainties surround the feasibility of remediation of different soil systems,

which cannot always be realised to the extent that the original state of the ecosystem is re-established (Figure 1.1). Many anthropogenic impacts on ecosystems are irreversible and long time periods have to pass to repair the damage or in the case of soils, centuries are needed for soil re-formation. The SOC lost while converting forest or grassland into cropland, for example, does not necessarily recover with re-conversion of land-use (Smith et al., 2016). In general, we lack a good understanding of the carbon accumulating potential of different soils in different places, which also prevents tailored best practice approaches for specific soils.

Whilst uncertainties surround the efficacy of new management to increase soil C, it is widely believed that soil C increase leads to other benefits to soil ESS such as enhanced water retention and biodiversity (Haddaway et al., 2017). Undoubtedly, minimum tillage can increase topsoil C, which can therefore lead to other benefits, though again these are poorly quantified. In particular, increased soil C is proposed to enhance soil microbial activity which can lead to enhanced ecosystem services (Vries et al., 2012; Kaiser et al., 2016). There is hence a desire to quantify the biological benefits of new management approaches, though to date there is no consensus on which organisms are promoted, likely due to the diversity of different soils and applied managements, and past difficulties in studying soil microbes. Since soil C accrual is known to take a long time, defining biodiversity indicators may also provide early indicators of restoration success, and to further optimise soil remediation practice. It is known that soil ESS are provided and maintained by soil biodiversity and thus, sustainable agriculture should target at increasing biodiversity in soils and cropping systems. Future regulation of ecosystem services urgently requires better indicators for monitoring soil health in natural and recreated land systems. Furthermore, bioindicators which validate the efficacy of the new agricultural practices have to be established, to ensure no natural, human, nor financial resource will be wasted on ineffective management practices.

1.2 Soil biological diversity - taxonomic and functional richness above and below ground

During the past decade, soils and the manifold lifeforms therein have been the subject of increasing interest from public,industry, political and scientific perspectives. Several soil initiatives were launched incorporating both local and global projects, starting with the World Soil Day launched during the UN General Assembly 2014 with support of the Food and Agriculture Organisation (FAO), implemented to raise awareness of soils as resource, followed by the Global Soil Week, aiming at sustainable land management by connecting policy-makers and scientists. This was followed up with the International Year of Soils in 2015 (FAO) and the UN Global Forum on Food Security and Nutrition in 2018, which underlined the importance of soil (biology and diversity) in enabling human life and well-being (Orgiazzi, Bardgett, and Barrios, 2016). Dominati, Patterson, and Mackay, 2010 pointed out the importance of soil biodiversity in providing soil ecosystem services and natural capital and it has been suggested to be a soil health indicator itself (Ritz et al., 2009).

Several of these initiatives have been aimed at the public with a goal of increasing the general awareness of soil biodiversity and its importance to soil health. Additionally, from a scientific perspective there has been an increasing number of studies aiming to empirically demonstrate the role soil biodiversity plays in delivering specific soil ecosystem services. These studies follow from an extensive literature exploring in above ground habitats the relationships between biodiversity and ecosystem resilience and multifunctionality (Naeem et al., 1994; Grime, 1997; Tilman et al., 1997; Bengtsson et al., 2000; Weisser et al., 2017). Such studies, based typically on experimental manipulation, have shown that higher richness of plants supports more plant biomass, and greater resistance and resilience to stress; though there are uncertainties as to whether this is a direct diversity effect (complimentarity) or simply that higher diversity supports more species capable of performing enhanced function (compositional effects). However to date, the complexities of the soil habitat and difficulties in quantitatively characterising soil organisms means that the functional significance of soil biodiversity in regulating soil ecosystem services remains

far from resolved.

Biodiversity in general, is assessed as species abundance, community structure or functional diversity. Traditional ecological diversity theory describing how diversity may support functionality is problematic to transfer to soils due to the complex interactions of highly diverse soil organisms from all trophic levels and the very heterogeneous characteristics of soil properties even at small scales. This means linking soil biodiversity to functionality through observation and survey is difficult, and experimental manipulation to prove causation is technically difficult to perform at realistic levels of diversity. Additionally, there are numerous fundamental methodological and analytical difficulties in measuring and quantifying soil diversity.

Direct observation of diversity and activity is troublesome given the nature of the soil matrix, which often means extraction is required which may not be fully representative. Traditionally, soil biodiversity was investigated on the macroscopic scale, focusing on macro- and mesofauna. For example, pitfall traps are common tools in past and current scientific studies on beetles (Paoletti et al., 1991; Eyre, Lott, and Garside, 1996), and Tullgren funnels are commonly used to assess smaller soil invertebrates. For earthworms numerous methods exist including digging and manual extraction or gassing with chloroform to force live organisms from the soil Macfadyen, 1961. Extraction methods also exist for nematodes, which then require laborious microscopic identification (LI et al., 2005), ants (De Bruyn, 1999) or biological structures (earthworm burrows, casts). A disadvantage of methods which require active dynamic movement of the organisms, is that they are difficult to apply in a large scale like national monitoring programs, because they have to be collected after two weeks in the field and are laborious and challenging to analyse in large numbers, due to their short storage time compared to the shelf life of dry or frozen sample material (Ritz et al., 2009). Additionally, these methods are known to bias estimates of diversity due to different behavioural patterns in response to the extraction method (Geisen et al., 2019).

The challenges of linking soil biodiversity to ecosystem services are therefore great, even for larger soil organisms. However, these challenges are amplified when considering soil microbial biodiversity. Considering soil nutrient cycles and organic

matter dynamics, bacterial and fungal communities are the primary agents in transforming plant derived organic matter (root exudates and litter (Figure 1.2)) via decomposition processes. However it is only since the advent of molecular technologies that we have begun to appreciate the true diversity and ecology of these important organisms, since their microscopic size has previously posed numerous technical challenges. Previous attempts at evaluating soil microbes in the context of land-use change or soil "health" were reliant on "black box" approaches such as laborious microscopic enumeration, cultivation, and gross measures of biomass determined by chloroform fumigation or from proxies such as basal soil respiration and substrate degradation (Geisen et al., 2019; Pulleman et al., 2012). These methods, whilst still in use today, provide no detail on specific responsive taxa or biodiversity responses in general. Fortunately with the advent of molecular technologies, soil microbial biodiversity can now be efficiently characterised leading to numerous breakthroughs in our understanding of soil microbial ecology and our appreciation of their roles in driving soil processes which contribute to soil ecosystem service delivery.

1.2.1 Microbiology: Importance of small things

Microbial communities, mainly composed of bacteria, archaea and unicellular eukaryotes like algae, protists and fungi are omnipresent on this planet, making up the second largest pool of biomass after trees. Generous estimates say, that one gram of soil can harbour several thousand bacterial and fungal species (Bouchez et al., 2016), and as such they represent the most diverse and numerous organisms in soil systems. Microbes, together with other organisms in soil, are members of the three functionally important groups in soils. Firstly, they act as chemical engineers (Kibblewhite, Ritz, and Swift, 2007), decomposing organic compounds and circulating nutrients for plants, degrading toxic compounds and mitigating pollution. Secondly, they are biological regulators, providing a food source for higher organisms in the soil foodweb, but also are involved in predation and parasitism activities which control population dynamics of other organisms in the plant-animal-microbe system (Figure 1.2). Alongside earthworms and larger animals, microbes are also ecosystem engineers as they can affect the physical properties of the soil matrix (Saccá et

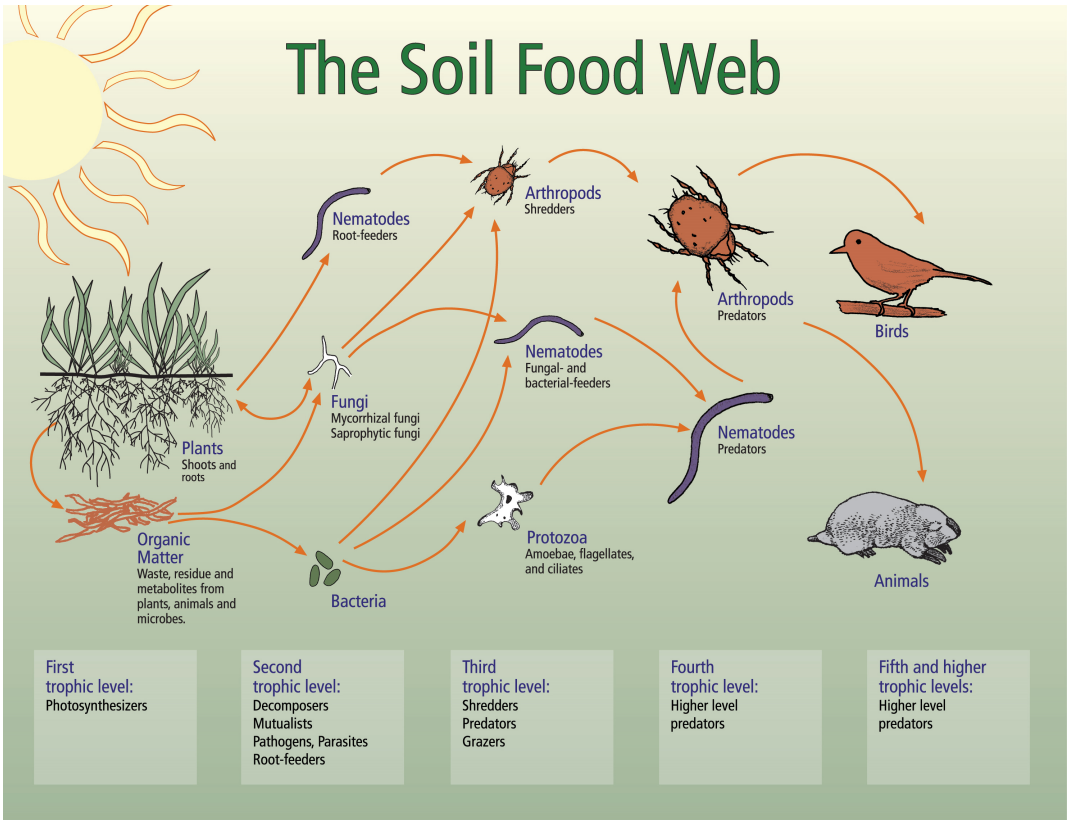

FIGURE 1.2: **Soil Food Web.** Photosynthetic active primary producers fix atmospheric carbon, which is then transferred and transformed by primary consumers (composed of decomposing bacteria and fungi and protozoan microorganisms). While mutualist bacteria and fungi enhance plant growth, pathogenic and parasitic microorganisms cause disease. Shredding earthworms and macroarthropods break down plant material and maintain soil structures. Higher level predators are especially important in regulating populations. Picture credits: United States Department of Agriculture.

al., 2017). Microbial excretions and necromass act as binding agents, substrates and weathering agents, which can shape soil structure,stability and porosity influencing wider soil hydraulic properties (Dominati, Patterson, and Mackay, 2010).

1.2.2 Specific ecosystem services delivered by soil microbes

A number of ESS are delivered by soils, many of those by microbial communities, which are summarised below (as suggested by Saccá et al., 2017):

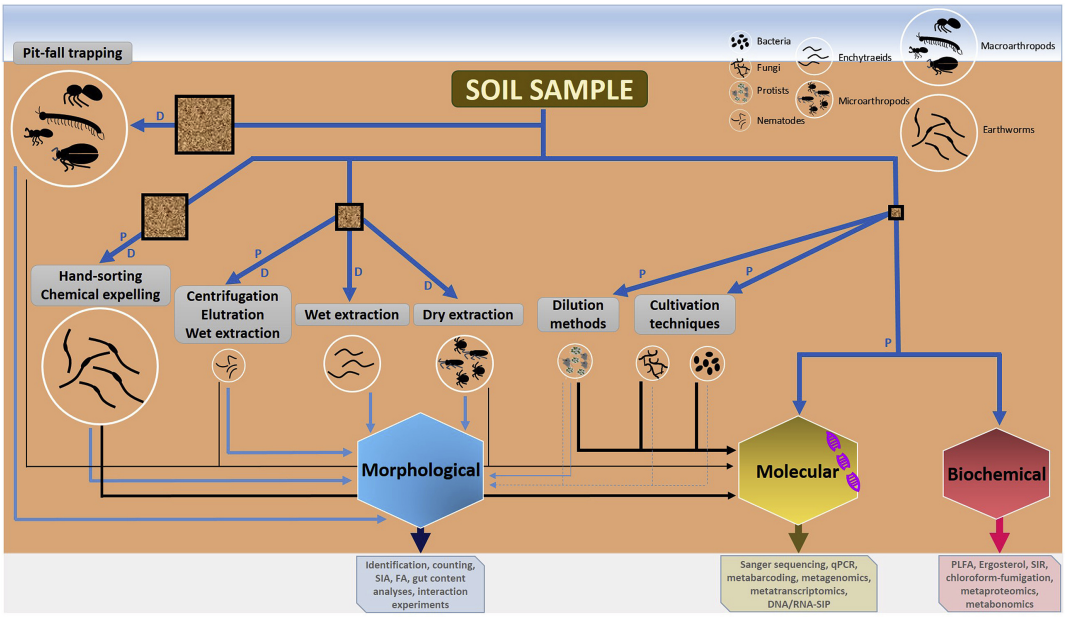

FIGURE 1.3: **Overview of general soil biodiversity and applied methodology**. Techniques to study each group of soil biota include morphological, biochemical and molecular methods. Sampling sizes differ between groups of organisms (brown filled squares) that need to be considered. Bottom part in grey shows subsequent quantitative and qualitative methods to study taxonomic and functional biodiversity in soils. Circle size indicates the size of the targeted organisms. Extraction approaches are split into those that depend on dynamic (D) movement of organisms and passive (P) methods that capture both active and inactive organisms. SIA: Stable isotope analyses; FA: Fatty acid analyses; SIP: Stable isotope probing; SIR: substrate induced respiration). Adapted from Geisen et al., 2019.

Regulating services

Regulating services provided by microorganisms in soils mainly relate to climate, water purification, disease control and remediation of xenobiotic pollutants.

The *global and local climate* is heavily regulated by soil prokaryotes, as they influence the C storage (capacity) of soils via formation and stabilisation of SOM from labile litter into their biomass, but also emitting greenhouse gases CO_2, CH_4 and nitrous oxide N_2O during respiratory activities (Oertel et al., 2016; Lal, 2004a; Bardgett, Freeman, and Ostle, 2008).

Including filter and storage functions of soils, the financial value of *water regulation* is estimated 16 billion Euros yearly, for Europe alone (La Notte et al., 2017b). Here, the metabolic diversity of bacteria plays a crucial role in degradation of pollutants.

Furthermore, they are involved in the service of flood and drought mitigation by actively stabilising soil structure and improving soil hydraulic properties, drainage and macroporosity (Dominati, Patterson, and Mackay, 2010).

Biological Disease and Pest Regulation has potential to replace synthetic agrochemicals with microbial pest control. This service is provided by a multitude of bacterial and fungal species in form of a) direct antagonism against, b) competition with or c) stimulating defense strategies in host plants against harmful pathogens (Ciancio, Pieterse, and Mercado-Blanco, 2016). The chance of soil borne pathogens or invasive species to establish is lower in soils with a stable soil community with an even species distribution (Elsas et al., 2012; De Roy et al., 2013).

Regulation of pollution and waste material via degradation, transformation, (im)mobilisation in microbial metabolism and co-metabolisms combats not only soil contamination as a threat to soil functionality, but protects furthermore the ESS water purification and plant primary production (Saccá et al., 2017). Polluted soils select for resistant species, which perform the natural attenuation in bulk soil and rhizosphere, named bioremediation and phytoremediation. This process can be enhanced by the addition of nutrients and oxygen,or inoculation with selected or engineered strains, but the transfer of microbial communities from lab conditions into real agricultural settings remains a challenge (Wenzel, 2009; Glick, 2010; Raskin and Ensley, 2000). Endophytic fungi were shown to degrade polyester (Russell et al., 2011), giving hope in face of the massive problem of plastic pollution worldwide.

Supporting ESS

Soil microbial communities are at the core of soil formation and the supporting ESS nutrient cycling, water cycling, plant primary production and soil biological activity (Saccá et al., 2017; Dominati, Patterson, and Mackay, 2010). The habitat that soils provide to numerous species is at the same time shaped, structured, transformed and detoxified by the multitude of microbial compounds and activities. These are primarily provided and maintained by bacterial and fungal chemical engineers, whose very own elemental composition of their biomass drives nutrient and organic matter

(re)cycling in soils (Spohn, 2016; Zechmeister-Boltenstern et al., 2015). In doing so, soil microorganisms support plant growth and diversity by enhancing eg. phosphorus availability, one key limiting factor of the vegetation in natural and agroecosystems (Jones and Oburger, 2010) or fixing atmospheric nitrogen either in symbiosis with leguminous plants or as free-living bacteria (Jones et al., 2016). Fungi as well as bacteria were shown to produce Fe-chelates (ie. siderophores), where especially the Actinobacteria (Streptomyces) are promising supporters for phytoremediation of heavy metal contaminants and biofertilization (Wenzel, 2009; Glick, 2010; Raskin and Ensley, 2000).

Provisioning ESS

The provisioning services delivered by soil microorganisms include the products food, clean water, fibre, raw materials (peat for fuel). Single Cell Protein (SCP) are edible unicellular organisms, including yeast, algae, bacteria and other fungi for human consumption, as a replacement for meat, as nutritional supplement or in animal feed (Trinci, 1992; Nasseri et al., 2011). Filamentous fungi like the ascomycete *Fusarium venenatum* create a structure in mycoprotein Quorn Foods (TM), which is comparable to the texture of meat products and depleted in amino acids and RNA, thus reducing the risk of allergies upon consumption. Moreover, soil microorganisms provide a unique genetic diversity and their manifold antimicrobial compounds, enzymes and metabolites are potential pharmaceutical and chemical resources for industrial use. The family Streptomycetaceae are source of one third of the currently produced antibiotics (Rosenberg et al., 2014).

Physical support and building ground for infrastructure is even provided by those soils, which are completely infertile. Some microorganisms can remediate cracked bentonite soils with their ability to produce biocement (Guo et al., 2018) and can serve as a tool to restore ancient artwork (Barbabietola et al., 2016), thus additionally contributing to the cultural ESS provided by soils.

1.2.3 Potential use as bioindicators

Biological indicators are species, that are used to trace an ecosystems' health, for example lichens in air quality assessments or macroinvertebrates in surface water monitoring as implemented in the EU Water Framework Directive. In the ecological context, they are of use to assess an ecosystems state, in monitoring programs of protected natural areas as well as to track the progress of conservation efforts. Bioindicators should be selected according to seven criteria: They should be meaningful, standardized, measurable and cost efficient, policy relevant, scalable, simple and easy to understand, and accurate (Pulleman et al., 2012; Griffiths et al., 2016a). Due to the fact that microbial communities adapt quickly to environmental change, they can serve as early warning indicators of land-use change (Saccá et al., 2017; Hermans et al., 2017).

Microbial 1) abundance or biomass, 2) community structure and 3) activity indicate changes in soils. Importantly, none of the three features should be assessed on its own, as the interplay of microbial biomass quantity and functionality remains a conundrum in relation to ESS. Instead, the combination of phenotypic, genotypic and functional soil microbial properties improves their indicative value, (Pulleman et al., 2012; Griffiths et al., 2016a), for example when relating respiration or enzymes to microbial biomass. Due to the species rich and highly interconnected trophic levels in soil life, there are functionally redundant species, whose extinction does not necessarily lead to a reduction in ES functioning. An exception are essential 'keystone species', whose presence is crucial for other species in the ecosystem and whose loss is disproportional deleterious for community composition and functioning (Paine, 1995; Berry and Widder, 2014). The use of co-occurrence networks revealed microbial taxa, which are strongly connected with others in various environments, but reports on soil microbial keystone taxa are very limited (Liddicoat et al., 2019; Banerjee et al., 2019). Indicator taxa and keystone taxa reflect two different microbial indices, with indicator taxa being exclusively abundant across all samples (fidelity) under a particular habitat, in contrast to keystone taxa being hub nodes in a network algorithm (Dufrene et al., 2011; Berry and Widder, 2014; Banerjee et al., 2019).

1.2.4 Previous challenges of assessing microbes

Functional assays in solid agar or liquid cultures have for a long time been used to assess microbial metabolic potential activities, ranging from substrate degradation, nitrogen fixation, siderophore and phytohormone excretion, to assays testing their resistance to xenobiotics and extreme environmental conditions. This approach perfectly links taxonomy of one species with soil function, but the realistic activity of the studied organism in natural soils might differ to that in pure mono-culture because of the manifold interactions in complex communities on top of those interactions with environmental factors. Again, only those microbial metabolisms are taken into account, where the organism is able to thrive under laboratory conditions. Until the advent of molecular biology with high throughput parallel sequencing platforms and the radical reduction of associated costs, soil microbial ecology studies were mainly based on culture dependent or functional methods. The problem with cultivation based methods is that apparently only a low proportion of less than 1% of all soil microorganisms grow on artificial media (Amann, Ludwig, and Schleifer, 1995), leaving out a vast majority. Assessments of soil respiration or litter degradation rates deliver quantified community responses, but no taxonomic information, hence a combination of methods needs to be put into practice, which account for microbial identities and their specific responses in the natural soil environment and in interaction with other soil organisms.

1.3 Molecular approaches for soil biodiversity assessments

1.3.1 History of molecular soil ecology

Soil molecular ecology seeks to define general patterns of microbial responses to environmental factors and change. The more detail we have in our understanding of how specific microbial populations and their functions change in response to the environment; then the better we can predict ESS responses to change, as well as optimising the exploitation of the newly discovered species, compounds and pathways for a variety of purposes. The effect of land-use on soils (and soil microorganisms)

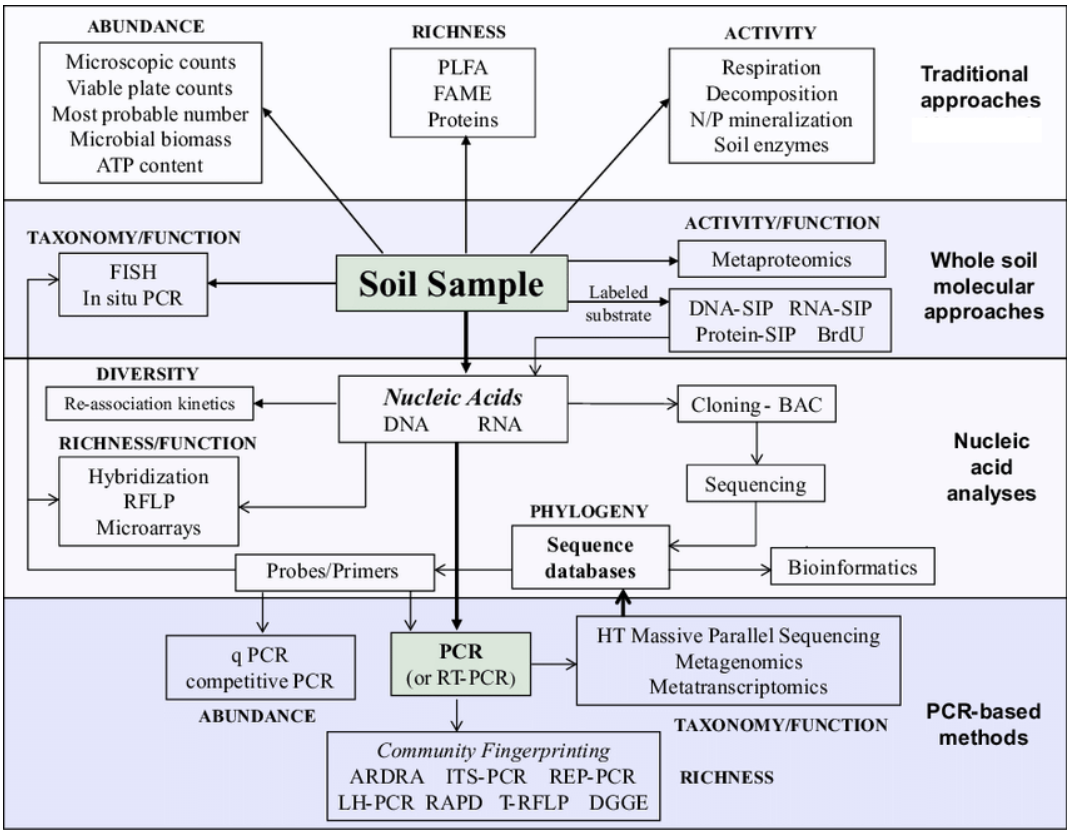

FIGURE 1.4: **Overview of traditional and novel molecular approaches investigating soil microbiology (adapted from Thies, 2015).** Soil microbial communities can be investigated in their 1) abundance/biomass, 2) diversity/richness and 3) metabolic activity, preferentially in a combination of the three to achieve a more holistic understanding of biota and the ecosystem services they deliver. Abbreviations: FISH - Fluorescent in-situ hybridisation, PLFA -Phospholipid fatty acid analysis, RFLP - restriction fragment length polymorphism, SIP - Stable Isotope Probing

has been investigated for decades, but traditional methods based on fungal and bacterial biomass only delivered quantification at a very broad taxonomic level (Bardgett, Hobbs, and Frostegård, 1996; Potthoff et al., 2006; Fierer and Jackson, 2006; Vries et al., 2012; Thomson et al., 2015). Microbial biomass was traditionally assessed using chloroform fumigation extraction (CFE) (Jenkinson and Powlson, 1976), which is based on *in situ* cell lysis through fumigation and simultaneous extraction of C and N contents derived from microorganisms. Unfortunately, CFE suffers from several methodological problems like high variance compared to other quantitative methods, which are now available (Zhang et al., 2017), but most importantly gives no insight into diversity or taxonomic identity. To overcome these limitations, methodologies were developed to assess microbial phospholipid-derived fatty acids (PLFA)

extracted from soils to provide microbial diversity and biomass measures. PLFA's are considered taxonomy specific microbial cell membrane biochemical markers due to distinct alkyl chains, which are known to discriminate different taxa. As PLFAs degrade quickly upon cell death, the acids are thought to characterise the viable microbial community (Hirsch, Mauchline, and Clark, 2010). Developed in the 1980s, and still in use today, PLFA has been extensively used to address many soil microbial ecology questions (White et al., 1979; King, White, and Taylor, 1977) and has been applied to reveal correlations of soil chemistry and microbial diversity under land-use change (Bardgett, Hobbs, and Frostegård, 1996; Potthoff et al., 2006; Stark, Männistö, and Eskelinen, 2014).

It is noteworthy that PLFA provided the first proposal of a universal soil microbial bioindicator, as it has been demonstrated that the ratio of fungal to bacterial biomarkers can be related to soil nutrient status and therefore inform on management intensity effects on soils. Indeed it is proposed that the dominance of fungi in low nutrient/extensively managed systems is functionally relevant and characteristic of conservative nutrient use life histories; whereas bacterial dominance in more intensive systems could represent exploitative and possibly less efficient life histories (Bardgett, Hobbs, and Frostegård, 1996). While the generality of these assumptions is still subject to debate, this represents the first attempt to link more detailed characterisation of the soil microbial communities with specific functional attributes of relevance to soil ESS delivery. Soil microbiota is largely driven by abiotic factors, with land-use influencing these factors.

1.3.2 Molecular approaches to assess soil microbial biodiversity

Molecular approaches for the detection of microorganisms in soils based on genetic marker genes have been in development since the early 1990s. DNA extraction from soil was initially challenging due to the high content of co-extracted humic acids, but improved methodologies were initially developed for research using PCR to track the fate of genetically modified microorganism released to the plant soil system (Cullen and Hirsch, 1998). Concurrently, advances in sequencing technologies and the adoption of the ribosomal RNA (rRNA) gene as a phylogenetic marker for

microorganisms led to an explosion in studies exploring the microbial biodiversity in soils. Initially, these studies involved laborious cloning and Sanger sequencing, meaning only a limited number of samples could be assessed in any study. However, the results of these early studies revealed that soils contained large numbers of previously undescribed taxa.

In the late 1990s/early 2000s rapid electrophoretic profiling techniques were developed which permitted a greater number of samples to be assessed in any one study, though with limitations as to the amount of taxonomic information which could be retrieved. PCR based methods to assess soil microbial richness in such a community fingerprint were amongst others denaturing gradient gel electrophoresis (DGGE), terminated restriction fragment length polymorphism (T-RFLP) and Amplified Ribosomal DNA Restriction Analysis (ARDRA) (Thies, 2015) (see overview of traditional and novel molecular approaches in Figure 1.4).
Later, the advent of next-generation sequencing technologies allowed the multiplexing of larger numbers of samples on individual sequencing runs, enabling unprecedented access to the soil microbial community or "microbiome" through the use of so-called "metagenomics" or "metabarcoding" techniques. The terms metabarcoding and meta-genomics are often used simultaneously, although they are referring to different methodologies and assess different varieties of diversity. Metabarcoding typically assesses taxonomic biodiversity via sequencing of a target gene (amplicon), answering questions about identity, distribution, dynamics and phylogeny of the target group. The name refers to DNA fragments which are used like barcodes to delineate genetic species (Hebert et al., 2003). This method is applied not only in soil microbiological context, but can also be used in higher organisms such as animals and plants. Metagenomics, more typically relates to the characterisation of the broad suite of functional genes present within a sample. Such methods also termed "shotgun" sequencing assesses randomly cut DNA fragments in association with functional gene annotation to reconstruct the genetic potential in communities.

It is notable that even though NGS/metabarcoding is the current gold standard

way of assessing soil biodiversity, the assays only provide relative abundance measures, and other approaches have also been developed to provide more quantitative measures. A method to quantify specific microbial populations is Fluorescent *in-situ* Hybridisation (FisH), which makes use of dye labelled probes, that are incorporated into microbial RNA inside the cell. It offers microscopic visualisation of taxonomic groups and their co-location, thus providing hypothesis building on ecological relevant questions related to species co-existence and competitive patterns in the environment. However, this approach is limited in that it is difficult to apply in soils, and requires considerable optimisation of probes to quantify specific taxonomic groups. Other molecular methods to directly assess soil microbial biomass include qPCR assays with targeted primers for different taxonomic groups (typically based on 16S rRNA) (Zhang et al., 2017) or functional genes. Typical functional markers used in soil studies include amoA and nifH which are involved in microbial nitrogen cycling and have been used to quantify functional responses to land-use change (Hayden et al., 2010).

1.4 Application of molecular approaches in soil ecology

Land management effects on soil microbial communities have been assessed at a range of scales. While large-scale studies in the global or national context revealed broad drivers of microbial structures (Thompson et al., 2017; Lauber et al., 2008; Lauber et al., 2009), local scale studies detected distinct responses of specific bacterial and archaeal taxa to management change (Zhalnina et al., 2013). An international consortium called the scientific community to sequence the metagenome of soils globally in the TerraGenome project (Vogel et al., 2009), similar to the Human Genome Project. Moreover, the Earth Microbiome Project developed standard protocols to sample bacterial communities from all kinds of environments to assess their biogeographic principles at an unprecedented scale in 2010 (Gilbert, Jansson, and Knight, 2014; Thompson et al., 2017).

Soils are amongst the most diverse habitats on earth, but the reduced costs with

high throughput technologies enabled the scientific community to map soil diversity at various scales in a comparable, standardised manner. Global biodiversity assessments crossing all phylogenetic groups and trophic levels aim to create an inventory of life in soils (Gilbert, Jansson, and Knight, 2014; Tedersoo et al., 2014; Thompson et al., 2017; Bahram et al., 2018; Delgado-Baquerizo et al., 2018). Molecular biology possesses tools to map soil microbial biogeography across landscapes, revealing ecological responses and patterns, as well as drivers of diversity and functionality (Griffiths et al., 2016c; Fierer, Bradford, and Jackson, 2007; Dequiedt et al., 2009; Martiny et al., 2006). However, land-use intensity effects on bacterial communities are difficult to evaluate in regard to SOM changes at this broad scale, as especially bacterial communities are structured along environmental gradients like soil pH (Griffiths et al., 2011). The land-use driven changes in edaphic properties can hence cover interactions of microbial taxa with SOM and land-use intensity (Lauber et al., 2008; Thomson et al., 2015). As mentioned above, soil microbiology provides valid indicative information of change in environmental conditions. In soils, this was shown for a change in physico-chemical properties (Griffiths et al., 2016c; Hermans et al., 2017; Lanzén et al., 2015), climate (Marilley, Hartwig, and Aragno, 1999; Lanzén et al., 2016; Zhao et al., 2016) and land-use (French, Tkacz, and Turnbull, 2017; Liddicoat et al., 2019).

Additionally to indicating changes and disturbance in ecosystems, soil microbial communities are actively participating in restorative processes and soil remediation and novel molecular tools offer firstly, tracking of these processes and secondly, detailed insights into the metabolic pathways involved. This could be in a context of microbial supported phytoremediation of toxic compounds (Glick, 2010), monitoring conservation efforts in protected areas or enhancing soil organic carbon stability and stocks (Cotrufo et al., 2013; Cotrufo et al., 2015; Kallenbach, Grandy, and Frey, 2016) via either successful manipulation of the microbiome or management practice, which supports an increased activity and abundance of such beneficial organisms.

Correlations between the communities of different taxonomic groups are observed in both, ecological surveys and experimental studies of eg. plant communities and communities from other domains of life (Ramirez et al., 2017; Delgado-Baquerizo

et al., 2018; Delgado-Baquerizo et al., 2020; Prommer et al., 2019). This is partly explained by the direct interactions between these groups on the one side, but also by the similar response of those groups to environmental factors and land management. Though there might be no direct linear relationship between species richness in above ground vegetation and below ground communities, there are clear patterns in community assembly and co-occurrence across the domains of life observed.

A clear separation of biotic from abiotic effects on soil biology and their specific, quantitative contribution in ESS delivery could help to predict land-use and climate change impacts on ES functionality and hence improve sustainable management.

1.4.1 Understanding the microbiome for crop and soil health

In smart agriculture, synthetic communities were shown to suppress diseases in crops (Hu et al., 2016) and stimulate plant growth with their hormones and secondary metabolites (Backer et al., 2018; Toju et al., 2018). Molecular methods allow us to select and apply targeted microbial taxa and/or the stimulating compounds and understand plant-soil-microbe interactions induced by these signalling substances. Thus, microbial inoculation and management practices that enhance crop beneficial organisms and suppress pathogenic ones are promising agents for sustainable intensification of agriculture. Microbial induced improved plant nutrient use efficiency could tremendously change agriculture with lower fertiliser requirements and reduce nitrate leaching and make land useful for agriculture which is so far not considered to be fertile.

Molecular microbiology methods allow the monitoring and design of specific microbial communities to be transferred as an inoculum to improve soil functionality and crop health. Firstly, DNA sequencing helps with the identification of taxa, which contribute to plant and soil health. Secondly, microbial taxa which are responsive to environmental changes offer potential as indicators to track the efficacy of novel land management, which then allow optimal fertiliser application (rates) and a selection of appropriate crops and pesticides. Artificial or synthetic soil microbial communities (microbial communities selected and cultured in laboratories to be then applied

to a host plant) can be used to promote plant performance in their nutrient acquisition, eg. phosphorus solubilisation (Jones and Oburger, 2010), disease suppression (Penton et al., 2014), increased stress tolerance and higher crop yields. The ever-decreasing costs for high-quality eDNA sequencing may allow regular control for a healthy soil microbiome in the mid-term future and also ensure the efficacy of land management in terms of survival and performance of the applied synthetic microbial community. Furthermore, enzymes involved in litter or toxin degradation can be assessed in detail and respired volatile organic compounds and other metabolites defined and characterised for industrial or research use.

1.4.2 Linking diversity to function

With the new methods to assess and manipulate below ground diversity and the corresponding functional assays (Figure 1.3, it is now feasible to follow the effects of land-use change on soil processes and understand the explicit role of biodiversity, and more specifically, the role of distinct taxa in delivering and securing such processes. Microbial communities can now be determined from smallest amounts of soil and scaled up and predicted onto the landscape scale. However, a characterisation of diversity does not allow us to infer function, as the majority of organisms is of unknown functionality. The variance of diversity in soil microbial communities at different scales could help to explain variation in soil processes in different soil types and climates. It is therefore an objective of this thesis to determine whether there are consistent responses of soil microbial communities to land-use change across different scales.

Many studies across the world examine land-use change effects on biodiversity and soils (Smith et al., 2016; Griffiths et al., 2016c; Lauber et al., 2008; Delgado-Baquerizo et al., 2020; Ramirez, Craine, and Fierer, 2012; Zhalnina et al., 2013; Hirsch et al., 2009; Potthoff et al., 2006). There is growing evidence for multiple diversity metrics, to be more precise: taxonomic, phylogenetic, and functional richness and mass ratio effects, to be simultaneously responsible for ES multi-functionality (Le Bagousse-Pinguet et al., 2019). If we want to account for this multi-level interactive complexity found in ecosystems, we need to move away from ecological monitoring in form of

counting taxa like static inventory. Instead, an assessment of biodiversity as combination of multi-dimensional complementary metrics, which take environmental changes into account, provides to be related to each other and soil ES functioning in a dynamic approach considering various scales. The novel molecular methods provide detailed insights of the diversity of microbial soil communities *in situ* and their sensitivity to change. Simultaneous assessment of community structure and function in soils is given with whole genome metagenomics and meta-transcriptomics (Urich et al., 2008), though associated costs and sample processing are limiting meta-transcriptomics application in large scales. Importantly, these methods only imply a functional potential, but not any actual process rates.

Geisen et al., 2019 claimed that the technological progress today enables us to combine sequencing data from various taxonomic and functional clusters and define their role in delivering ESS. So far, only one single study incorporated all the organismic groups *and* investigated them in the experimental microcosm scale, as well as global survey scale to draw multi-functionality relationships (Delgado-Baquerizo et al., 2020). This study found a positive relationship between diversity of single groups of organisms and the multi-diversity and furthermore higher soil ES multi-functionality, when assessed as plant net primary productivity, antimicrobial resistance control, pathogen control, nutrient cycling and OM decomposition.

A better understanding of the underlying microbial mechanisms in global element cycles and the role of microbial diversity therein could help to predict soil responses to climate and land-use change scenarios, leading to better soil ecosystem services models and thus improve information for policy and land owners. However, farmers want to know if this change in microbiota structure is going to pay out and policy makers require information on how it affects quantifiable ES. More generally, we need to translate our new microbial understanding into useful indicators to predict future environmental conditions in the face of climate and land-use change (Crowther, Averill, and Maynard, 2019; Bardgett and Caruso, 2020; Yin et al., 2020; Lanzén et al., 2016).

Today, new molecular methods enable us to identify microbial community members which are responsible for functional changes by following their distinct metabolic

pathways in combined approaches of DNA/RNA sequencing with SIP tracer experiments. Experimental microbial biodiversity gradients revealed loss of ES functioning with species loss (Griffiths et al., 2004; Bell et al., 2005). These diversity gradients were created by dilution-to-extinction approaches or transfer of microbial communities, but most importantly they assessed microbial diversity and ES functions simultaneously under controlled conditions. The contrary approach would be selective exclusion of microbial taxa by addition of specific antibiotic compounds, allowing to differentiate between fungal and bacterial contributions to soil functions, as an example. Whatever method is used to affect microbial diversity and community composition, simultaneous multiple measures are necessary to evaluate the effects on soil health. In comparison, the link between plant diversity and ES multi-functionality in terrestrial ecosystems was reported in global survey and field experiments, es well as in microcosm studies (Maestre et al., 2012a; Zavaleta et al., 2010; Maestre et al., 2012b; Le Bagousse-Pinguet et al., 2019). They found reduced ES multi-functionality related to carbon and nutrient cycling with diversity loss. This was assessed as enzymatic activities, metabolites, nucleic acids and plant available nutrients. A recommended approach for future ESS - biodiversity investigations, which consider microbial diversity, should therefore consider multidimensional diversity attributes and move away from single taxonomic perspectives (Le Bagousse-Pinguet et al., 2019).

An exemplar approach coupling genomics with soil functionality to tremendously improve informative quality, is the assessment of extracellular enzymes simultaneously with nucleic acids and microbial biomass. When investigating soil extracellular enzymes as a proxy for nutrient cycling, there are a couple of difficulties to consider. Firstly, the potential enzymatic activity is assessed under lab conditions which may account for stabilised enzymes, which would not be active *in situ*, after the soil was disturbed during sampling, transport and storage. Neither temperature nor pH of the assay buffer resemble natural conditions and are often ignored though being main factors driving enzymatic activity (Burns et al., 2013). Moreover, no information about the source of enzymes (ie. which microbial taxa produced it), nor production or turnover rates of enzymes are provided by soil extracellular enzyme

assays *per se* (Wallenstein and Weintraub, 2008). Coupling enzyme assays with genomics will deliver insights in actual OM decomposition and stabilisation processes and determine keystone species involved in these, but to complete the picture another functional assay accounting for biomass, soil respiration and eg. litter mass loss is required. Transcriptomics or targeted sequencing of functional enzyme encoding genes can furthermore give realistic information about enzyme production rates (Wallenstein and Weintraub, 2008). These methods are still emerging tools and being continuously further developed for the application in soil microbiology, but exciting new insights are awaiting molecular soil ecologists using such novel approaches.

1.5 Thesis Aims

Soil properties are incredibly variable depending on location, climate, pedogenesis and land-use. It is hence difficult to generalise functional responses of soil microbial communities to change, especially when soil physicochemical properties were different in the first place. In simple words, the microbial community in acidic soils will likely react different than the soil community in high pH soils. In order to manage land and natural resources in a sustainable manner, we need to define reliable indicators of change and relate them to specific functions delivered by soil ecosystems and furthermore assure these indicators are universal across soil systems. Though the composition of soil microbial communities is highly complex, it is now possible to assess the relative abundances of specific microbial taxa thanks to the technological advances in DNA sequencing and bioinformatics. However, it is necessary to establish if there is consistency in their indicative meaning and thus evaluate their appearance in data sets which have been recorded on distributed sites covering a variety of soil types and environmental factors. For predictions of synergies and trade-offs between agricultural intensification and natural ecosystem services, it is thus necessary to collect data accounting for different cropping and farming practices on top of the local distribution. The interactions between plant variety and soil microbiota are very specific and furthermore influenced by application of pesticides,

nutrients and mechanic disturbance. If there are still clear responses of universal indicators across systems, they could serve as early detectors of changes in SOM stocks/soil health and thus report success in sustainable management of soils.

Moreover, it is of interest to manipulate soils and the life within, in order to elevate microbial indicator taxa and assess their contribution to eventually changed soil functions. Once microbial key players in soil service delivery are determined in field surveys, they should be transferred via inoculation or supported by their favoured growth parameters in laboratory experiments. Only under strictly controlled conditions, it is then possible to quantify soil functions and microbial community composition in high resolution and hence elaborate the relations and contribution of microbial networks to ESS. Soil microbiota and the complex mechanisms governing ESS are often considered a 'Black Box' (Cortois and De Deyn, 2012) in the global biogeochemical cycles. Moreover, they are overlooked in biodiversity conservation initiatives (Guerra et al., 2020) though their fundamental contribution to ecosystem maintenance. Therefore, this thesis aims to quantify the effects of land-use change on soil microbial communities and their delivery of ecosystem services, with focus on carbon cycling. I will make use of a catchment scale survey to identify and validate microbial indicators of land-use change and test their specific responses to land management practices. This will allow an assessment of taxa, which are consistently responding to land-use change across widely distributed sites. These indicators will further be applied in a national scale farming context, testing the efficacy of land-use extensification and organic farming (Conservation Agriculture) in protection and enhancement of soil organic matter stocks and the enhancement of soil biodiversity. Underlying mechanisms involved in plant-soil-microbe interactions will be tested in the laboratory scale in a mesocosm experiment, to remediate the negative effects of long term intensive ploughing on soil chemical and biological properties.

The objectives of this thesis are to

- define molecular microbial indicators of land-use (change) in soil nucleic acids and extracellular enzymes, and explore their relation to C sequestration potential of soils and their wider role in nutrient cycling,

- test the recovery of such indicator molecules in a calcareous grassland restoration chronosequence,
- evaluate the effectiveness in soil carbon sequestration and biodiversity of Conservation Agriculture in British farm soils, based on the defined indicator organisms and topsoil organic matter contents
- explore the interactions of soil chemical properties, especially pH, with microbial diversity and carbon related ecosystem services,
- experimentally manipulate soils to enrich/deplete targeted members of microbial communities and establish their relative roles in delivering soil functionality.

Chapter 2

Bacterial and archaeal taxa are reliable indicators of soil restoration across distributed calcareous grasslands

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Authorship

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Author contributions

Richard Pywell designed the survey and carried out sampling and field work, Melanie Armbruster and Timothy Goodall carried out laboratory analysis and analysed the data with Rob Griffiths. Melanie Armbruster wrote a first draft and all co-authors contributed to the final version of the paper. Kate Fagan identified and surveyed the original sites.

2.1 Abstract

Land-use intensification can reduce soil carbon stocks and changes microbial community biodiversity and functionality. However, there is a lack of consensus on whether management consistently affects microbial biodiversity across geographic scales, and how this relates to altered soil function. From a regulatory and monitoring perspective, there is a need to identify functionally relevant indicators of land use in order to evaluate the progress of soil restoration approaches. We performed a landscape-scale survey of unimproved calcareous grasslands paired with local arable contrasts, and assessed the consistency of responses in a variety of soil, biotic and functional measures. In addition, adjacent grasslands undergoing restoration were assessed to identify soil microbial indicators of recovery. Organic matter content was consistently larger in grasslands than in arable fields, and increased with time in the restoring sites. Molecular comparisons of grassland versus arable soils revealed numerous bacterial, archaeal and fungal indicators, with more representatives of *Ca. Xiphinematobacter*, *DA101*, *Bradyrhizobium*, *Rhodoplanes*, *Mycobacteria* and *Mortierella* in old grassland soils, whereas Nitrososphaera, Sporosarcina and *Alternaria infectoria* were more abundant in arable soils. Extracellular enzymatic responses were more variable, with none of the eight investigated enzymes being consistent indicators of grassland or arable soils. Correlation analyses, incorporating the molecular and enzymatic responses across all surveyed soils, revealed that molecular indicators were more strongly correlated with soil organic matter increases with restoration of arable soils. Our results highlight that microbial taxa are among the most sensitive indicators of soil restoration, and we identify consistent responses of specific taxa to management across geographic scales. This discovery will be important for both the instigation and monitoring of soil restoration.

2.2 Introduction

Microorganisms play a major role in delivering soil ecosystem services, including nutrient cycling, soil aggregate stability, plant productivity and biodiversity (Fierer, 2017). For example, as plant pathogens or symbionts, soil bacteria and fungi can

significantly influence crop yields in agriculture, and recent evidence is emerging regarding the central role of microbes in increasing soil carbon stocks (Cotrufo et al., 2015; Cotrufo et al., 2013). Differences in land management are known to have strong effects on microbial biodiversity (Griffiths et al., 2011), yet we are still some way from synthesizing how land management affects the abundances of specific microbial taxa, precluding wider understanding of functional effects. Better understanding of the resistance and resilience of soil microbial communities and their functions for land-use change might provide novel approaches for future sustainable agriculture as well as for restoring ecosystems (Griffiths and Philippot, 2013). In addition, policymakers and land users require reliable indicators of soil function in order to monitor soil state and the efficacy of ameliorative practices (Orgiazzi et al., 2015; Stone et al., 2016).

Grasslands cover about one-quarter of the world's ice-free area and make up 70% of global agricultural land, storing 20% of global soil carbon (Smith et al., 2016). More than 90% of English and Welsh unimproved, species-rich grasslands were converted to more intensive agriculture between 1932 and 1984 (Ridding, Redhead, and Pywell, 2015). The associated cultivation has dramatically modified soil organic matter (SOM) stocks (Deng et al., 2016; Thomson et al., 2015).

To prevent further loss of soil C and vulnerable habitats, efforts have been made to restore degraded landscapes and abandoned fields to grassland in the UK (Bullock, 2011), but to date there has been little information on how soil C and wider microbial communities and features recover. Their ability to rapidly adapt makes microorganisms potential early indicators of succession during the regeneration progress (Bouchez et al., 2016; Griffiths et al., 2016b). Past research has identified that microbial biomass and activity is reduced under intensive arable management, and it is thought that intensification leads to a general reduction in fungi compared to bacteria (Emmerling, Udelhoven, and Schröder, 2001; Lauber et al., 2008; Nunes et al., 2012; Potthoff et al., 2006).

New molecular methods now permit a more detailed examination of the responses of individual soil microbial taxa (Hirsch, Mauchline, and Clark, 2010; Vogel et al., 2009), although we are some way from synthesizing whether there are geographic

consistencies in taxonomic responses to management. Identifying such taxa, and particularly those taxa associated with SOM content increases, will advance new functional understanding of the roles of microbes in soil processes, as well as providing functionally relevant indicators to assess soil recovery.

The effect of land management on soil microbial communities has been assessed at a range of scales, from local studies assessing the impacts of specific managements, to broader landscape-scale surveys. At the local scale, one study of bacterial and archaeal communities identified that across three sites there was some consistency in specific indicators of grassland versus arable communities (Zhalnina et al., 2013). This study found that specific archaeal taxa were associated with arable sites, whereas Bradyrhizobia were more abundant in grassland/abandoned arable fields. At the regional scale, a distributed study of bacterial and fungal taxa across arable and grassland sites focused on assessing broad diversity effects, but also noted key increases in dominant bradyrhizobial taxa in grasslands.

Notably, neither of these studies examined the specific relationships between these taxa and SOM. A critical issue in identifying microbes responsive to SOM changes has been identified in several studies examining intensification effects on microbial communities. Because soil microbes, and bacteria in particular, are primarily structured along gradients of pH (Griffiths et al., 2011), land-use-driven change in other edaphic properties can often obfuscate direct relationships between intensification, SOM and microbial taxa (Lauber et al., 2008; Thomson et al., 2015). It is, therefore, likely that constraining contrasts to land-use comparisons of soils of similar pH may help identify specific indicators relating to SOM and the lack of disturbance from cultivation.

We therefore seek to determine the consistency of microbial indicators across distributed sites in the south of England, each containing three land management contrasts. Each site selected comprised three contrasting land-use categories, including a contemporary intensively managed arable field, ancient grassland and a restoring former arable field established 3–65 years ago (Fagan et al., 2008; Wagner et al., 2019). These calcareous grasslands are typically characterized by high levels of plant and faunal diversity and are considered the most diverse habitats in Europe (Poschlod

and WallisDeVries, 2002).

Here, we specifically focus on calcareous soils to minimize wider confounding effects of soil pH on microbial communities, and consequently hypothesize that consistent microbial indicators of land-use change in pristine versus arable contrasts will be apparent across the distributed sites. Relatedly, across all soils assessed we hypothesize that microbial communities will be dominantly structured across gradients of organic matter and not pH. Finally, we predict that key microbial taxa found to be indicators of pristine grasslands will increase proportionally with SOM improvements through restorative management. The performance of microbial indicators will additionally be contrasted with enzymatic functional measures to test the utility of such metrics for informing on soil status under a restoration context.

2.3 Materials and Methods

2.3.1 Sampling sites

Fourteen undisturbed calcareous grasslands (henceforth "Pristine") were identified in the south of England 2.1, which were not ploughed, nor improved for grazing for at least 100 years (Fagan et al., 2008; Redhead et al., 2014). Arable fields near each site were used as a control or contrast, which is the land use that replaced the calcareous grassland. At each location, a reverting, ex-arable grassland ("Restoration") was sampled to test for the response of identified indicators to recovery over time. Both the Pristine and restoring grasslands were subject to livestock (sheep and/or cattle) grazing at low stocking density and without agricultural improvements. Details of actual stocking rates and grazing dates were unavailable. Dates when reversion of arable land to grassland started are based on past data, which investigated land-use history utilising historic maps (Fagan et al., 2008; Fagan et al., 2010; Redhead et al., 2014; Ridding, Redhead, and Pywell, 2015). Grassland age in the restoring fields differs strongly between sites, so that "Restoration" is not considered a defined land use or treatment. Instead, we focus on statistical comparisons between Arable and Pristine. To ensure comparable soil properties, the sample sites were situated on a chalk, lime-rich bedrock material, with the "Pristine" site classified as NVC habitat

Calcareous Grassland. Sampling was conducted in summer 2016, with plant cover assessed in five quadrats at each site, and co-located soil cores (20 cm depth, 5 cm diameter) sampled for further analysis. A subsample of each of the five cores was stored at -20°C for microbial diversity and enzymatic analyses. The remaining soil from each of the five cores was pooled for standard chemical analysis of SOM (as loss-on-ignition, 16 hr at 430°C), total C using the Walkley-Black method, total N, C to N ratio, Olsen's P, K, Mg (NRM Laboratories, Bracknell, UK) and pH (10 g soil in 25 ml distilled water).

FIGURE 2.1: Location of sampling sites on chalk-rich parent material in south England. At each site, a land-use contrast of unimproved grassland vs. intensive agriculture vs. reconverted, former arable grassland (3 to 65 years of regeneration time) was surveyed for plant assemblage, soil chemistry, soil bacterial and fungal diversity

2.3.2 Extracellular enzyme activity and bacterial biomass

Three of the five soil cores were randomly selected for extracellular enzymatic activity assays and the same soil solution was used to extract total DNA and measure bacterial biomass (see below). Potential activity of hydrolytic exoenzymes acetase (acetyl esterase, ACE), α-glucosidase (α -GLU), β-glucosidase (β-GLU), chitinase (N -acetyl-b-glucosaminidase, CHIN), phosphatase (PHO), sulphatase (aryl-sulphatase, SUL) and peptidase (leucine-aminopeptidase, LEU) was assessed with

methylumbelliferyl (MUB) and 7-amino-4-methylcoumarin (AMC) conjugated substrates (Sigma-AldrichCompany Ltd, Gillingham, UK). Enzyme assays were performed on 1.5 g of frozen homogenized soil mixed with 20 ml deionized water in sterile falcon tubes. Samples were shaken for 20 mins at 400 rpm to obtain a homogeneous soil solution; 30 ul soil solution was added to a 96-well microplate containing 170 ul substrate solution at 300 mM (saturated concentration). Reaction plates were incubated in the dark for 3 hr at 28°C with one fluorometric scan every 30 min (BioSpa 8 Automated Incubator, BioTek, Swindon, UK). Fluorescence intensity was measured using a Cytation 5 spectrophotometer (BioTek Swindon, UK) linked to the automated incubator and set to 330 and 342 nm for excitation and 450 and 440 nm for emission for the 4-MUB and the 7-AMC substrate, respectively. For each sample, three technical replicates (soil solution + substrate + water) and a quenching curve (soil solution + water + 4-MUB or 7-AMC) were measured. For each substrate, a control including the 4-MUB- or 7-AMC-linked substrate and water alone were used to check the evolution of fluorescence without enzyme degradation over the duration of the assay. All enzyme activities were calculated in [nkat], the amount (nmol) of catalysed product per second and normalized by g of dry soil (Marx, Wood, and Jarvis, 2001).

To assess bacterial biomass, 250 ul of the soil slurry was mixed with 750 ul water, centrifuged at 1000 g for 5 min, and 500 ul of the supernatant fixed with 500 ul 0.5 % paraformaldehyde solution for storage at -20°C. All samples were run using the Accuri® Flow Cytometer (Becton Dickinson UK Ltd, Wokingham, UK) in deep-well plates after SYBR Green staining and 5 min incubation in the dark as described in (Bressan et al., 2015).

2.3.3 Molecular analyses of microbial communities

For DNA extractions, a 200-ul aliquot of the soil-water slurry used for the enzyme analyses was transferred into 96-well plates and extracted using the PowerSoil® DNA Isolation Kit (QiagenLtd, Manchester, UK). Illumina 2-step amplicon sequencing was conducted according to the protocols of the Earth Microbiome Project (Thompson et al., 2017) (Thompson et al., 2017). In brief, amplicons were prepared using

established primers for the ITS regions GTGARTCATCGAATCTTTG and TCCTCCGCTTATTGATATGC (Ihrmark et al., 2012) and 16S rRNA regions (V4-5 region) 515f GTGYCAGCMGCCGCGGTAA and 806r GGACTACNVGGGTWTCTAAT, and PCR protocols (Walters et al., 2016) using high-fidelity DNA polymerase (Q5 Taq, New England Biolabs (UK) Ltd, Hitchin, UK). Amplicon sizes were determined using an Agilent 2,200 TapeStation system (Agilent Technologies LDA UK Ltd, Didcot, UK). For purification, PCR products were treated according to manufacturer's instructions with Zymo DNA Clean up Kit (Zymo Research Europe GmbH, Breisgau, Germany). In a second round of PCR, Illumina adapters were added and all samples normalized using the SequalPrep ™ Normalization Kit (Thermo Fisher Scientific Ltd, Altrincham,UK), pooled and concentration verified spectrophotometrically with Qubit (Thermo Fisher Scientific Ltd, Altrincham, UK). Illumina high-throughput sequencing was performed with MiSeq® Reagent Kit V3, which is capable of producing 2 × 300 bp paired-end reads (Illumina Ltd, Cambridge, UK).

Illumina sequencing output was analysed with DADA2 (Callahan et al., 2016) in R (R Core Team, 2017), to demultiplex raw sequences and trim paired sequences to uniform lengths. The core sequence-variant inference algorithm was applied with the DADA function to dereplicated data before paired-end sequences were merged and chimeras were removed. Taxonomic data were assigned from GreenGenes (DeSantis et al., 2006) for bacterial and UNITE (Koljalg et al., 2005) for fungal taxonomy. The 16S phylotype abundance table was rarefied to 4,590 reads, whereas the ITS table was rarefied to 2000 reads to account for differences in sampling depth, before assessing β-diversity in non-metric multidimensional scaling ordinations and running Permutational Multivariate Analysis of Variance (PERMANOVA) with the functions in vegan (Oksanen, 2008). Significant ($p < 0.05$) indicator phylotypes for Pristine grassland and Arable soil were determined using the indval routine in labdsv (Dufrene et al., 2011) and wider statistical analysis and visualization was performed in R version 3.6.0 using the packages ggplot2 (Hadley Wickham, 2016), circlize (Gu et al., 2014), labdsv (Roberts, 2019) and igraph (Csardi and Nepusz, 2006).

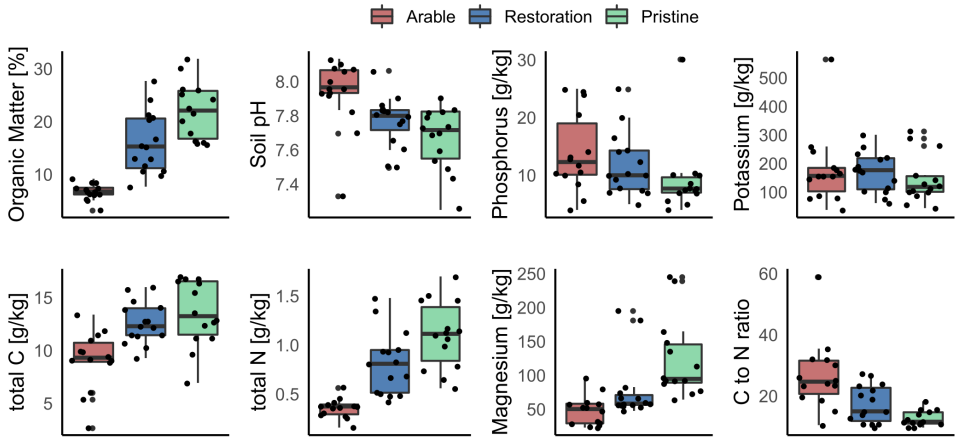


FIGURE 2.2: Boxplots of soil properties and plant available nutrients per land use across 14 sites. Arable soils are conventional croplands with elevated levels of P and greater C to N ratio. Pristine soils were not ploughed or fertilized for at least 100 years, but maintained as species-rich grasslands with high levels of SOM, C and N. Soil nutrient levels of ex-arable fields are recovering with time

2.4 Results

2.4.1 Soil properties

To assess the effects of land use on soil variables at each location, we quantified soil pH, SOM, P, K and Mg, as well as total C and total N, and present data grouped by management in Figure 2.2. SOM content in pristine grasslands was significantly greater than in arable soils, with a mean of 22.16 % and only 6.76 %, respectively (t -test, $p < 0.001$). Phosphorus determined by the Olsen method and soil C:N ratio were less in old grassland soil compared to Arable, whereas all other tested parameters, with the exception of potassium, were significantly greater in Pristine. With respect to pH, arable soils were slightly less acidic (pH 7.9 vs. pH 7.7 in pristine grassland, t -test, p -value 0.0016). All reverting soils showed attributes intermediate between grassland and Arable (Figure 2.2, Table 2.3).

Soil extracellular enzyme activities did not respond as consistently to land-use change as did the soil properties (Figure 2.3). From the eight evaluated enzymes only ACE and CHIN were affected by land use, whereas variance in PHO, hemicellulase (HEM) and β-GLU was completely independent from land use. Comparison

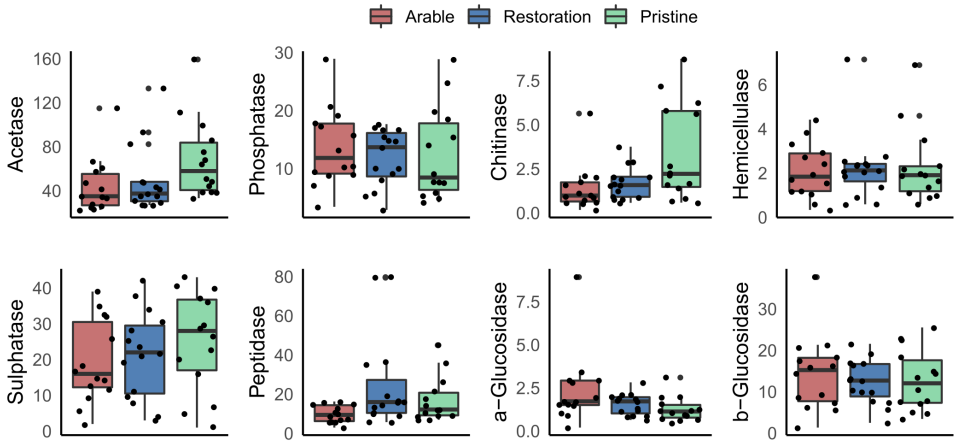


FIGURE 2.3: Eight hydrolytic soil extracellular enzymatic activities in nkat (nanomol substrate degraded per minute, normalized per gram dry soil) as response to land use in a calcareous grassland restoration chronosequence. Acetase, Chitinase, α- and β-glucosidase and hemicellulase activities are considered to be relevant for carbon compound degradation, whereas phosphatase (aryl-phosphatase) is involved in P cycling and peptidase (leucine-aminopeptidase) catalyses degradation of nitrogen compounds (peptides)

of Pristine and Arable soils show mean α-GLU was most active in Arable samples, but not significantly different between land-use categories (Table 2.4, p = 0.08). ACE activity increased with decreasing land-use intensity and was significantly stronger in Pristine than in arable soils (p = 0.048). CHIN and SUL mean activities were twice as high in Pristine soils as in Arable, with CHIN being significantly affected by land use (p = 0.024), whereas differences in SUL activities were not significantly different between land-use categories (p > 0.05). Interestingly, LEU showed more potential activity in Restoration sites than in pristine grasslands.

2.4.2 Land-use effects on plant and microbial community structure

Multivariate assessment of bacterial and fungal communities revealed samples grouped clearly according to land use, as assessed by non-metric multi dimensional scaling ordination of Amplicon Sequence Variant relative abundances Figure 2.4. The plant community ordination, based on presence/absence data from surveyed quadrats,

TABLE 2.1: PERMANOVA results of soil microbial community composition in bacterial, fungal and plant cover as a response to the land use types undisturbed grassland vs. cropland.

	Degrees of freedom	Sums Of Squares	Mean Squares	F value	R2	p
bacterial 16S	1	1.330	1.330	8.492	0.279	0.001***
Residuals	22	3.445	0.157		0.721	
Total	23	4.774			1.000	
fungal ITS	1	1.374	1.374	5.650	0.176	0.001***
Residuals	26	6.449	0.248		0.821	
Total	27	7.823			1.000	
plant cover	1	2.955	2.955	25.440	0.495	0.001***
Residuals	26	3.020	0.116		0.505	
Total	27	5.975			1.000	

TABLE 2.2: Linear fit of environmental variables to the non-metric multidimensional scaling ordination for bacterial (left) and fungal (right) soil communities. ACE = acetase, α-glu = α-glucosidase, β-glu = β-glucosidase, CHIN = chitinase, HEM = Hemicellulase, PHO = phosphatase, LEU = Peptidase, age = years since reconversion from arable to grassland, SOM = Soil Organic Matter content

bacteria						fungi					
	NMDS1	NMDS2	R2	p value			NMDS1	NMDS2	R2	p value	
SOM	0.97	0.26	0.85	0.001	***	age	0.97	0.24	0.65	0.001	***
total N	1.00	0.09	0.78	0.001	***	SOM	1.00	-0.03	0.53	0.001	***
age	0.99	0.14	0.66	0.001	***	CHIN	0.48	0.88	0.44	0.001	***
pH	-0.87	-0.50	0.66	0.001	***	total N	0.99	-0.12	0.44	0.001	***
moisture	0.83	0.56	0.62	0.001	***	Mg	0.82	0.58	0.40	0.001	***
C to N	-0.99	-0.14	0.60	0.001	***	C to N	-0.98	-0.18	0.38	0.001	***
Mg	0.73	0.68	0.53	0.001	***	pH	-0.85	-0.52	0.36	0.001	***
total C	0.89	-0.45	0.50	0.001	***	moisture	0.91	0.41	0.27	0.002	**
bact. biomass	-0.34	0.94	0.38	0.001	***	total C	0.77	-0.64	0.25	0.006	**
ACE	0.61	0.79	0.35	0.001	***	bact. biomass	-0.63	-0.77	0.24	0.008	**
CHIN	0.47	0.88	0.27	0.003	**	ACE	0.60	0.80	0.23	0.007	**
P	-0.50	-0.87	0.21	0.024	*	P	-0.96	-0.29	0.21	0.007	**
LEU	-0.11	0.99	0.16	0.048	*	HEM	0.39	0.92	0.13	0.063	.
α-glu	-0.89	-0.46	0.08	0.259		α-glu	-0.85	0.52	0.11	0.110	
PHO	-0.33	-0.94	0.03	0.581		LEU	-0.19	-0.98	0.06	0.110	
K	-0.80	-0.60	0.03	0.613		K	-0.68	-0.73	0.05	0.345	
β-glu	-0.78	-0.63	0.01	0.820		β-glu	-0.71	0.70	0.04	0.426	
HEM	-0.11	0.99	0.00	0.973		PHO	-0.97	0.23	0.02	0.643	

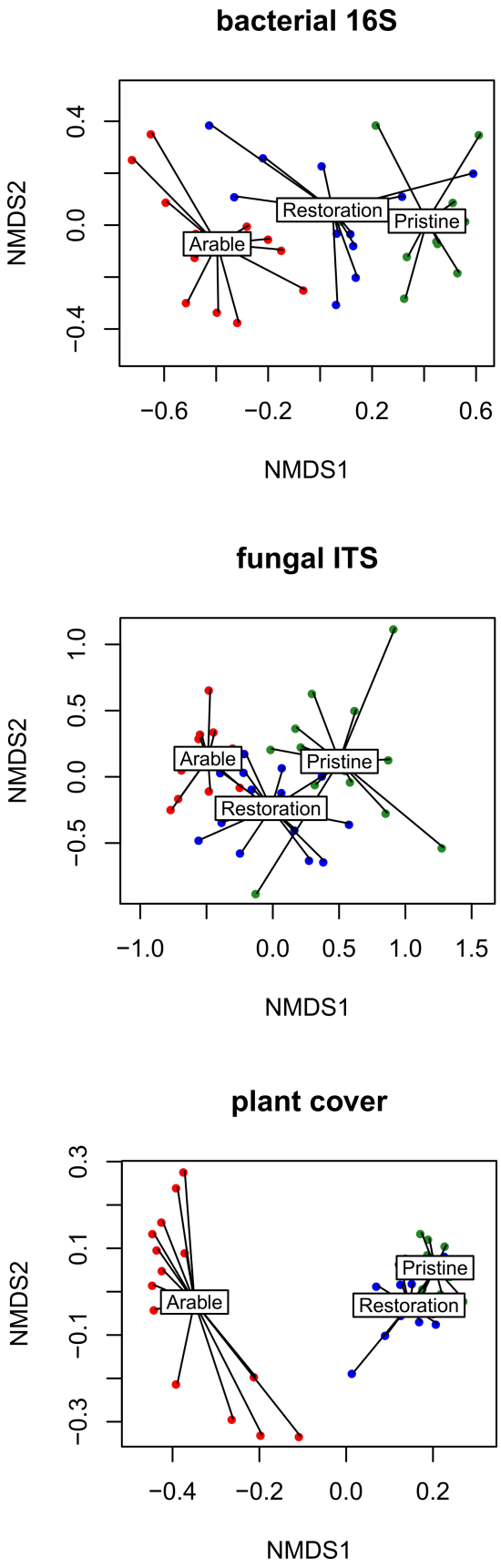


FIGURE 2.4: Non-metric dimensional scaling plots showing differences in microbial and plant community composition between treatments. Bacterial, fungal and plant communities were all significantly different in grassland compared to arable soils (PERMANOVA, $p < 0.01$), with restoration sites having an intermediate centroid

as expected showed that Arable communities were highly dissimilar to the grasslands. Further significance testing using PERMANOVA revealed all grassland communities were significantly different from arable land (Table 1, PERMANOVA $p < 0.01$, $F > 0.5$). Restoration sites were situated between grassland and Arable, and the variance within this group is likely to reflect different times since arable abandonment. We also fitted the soil chemical and enzymatic data to the non-metric multidimensional scaling (NMDS) plots to examine specific relationships with microbial community composition (Table 2). For both bacterial and fungal communities, SOM and age (time since cultivation) were highly related to community composition, and importantly, these variables were stronger than pH. In accordance with the results shown in Figure 2.2, enzymatic responses were more weakly associated with microbial communities, although it is noteworthy that CHIN was jointly the third strongest linear fit with fungal community structure.

2.4.3 Molecular indicators of land use change

Indicator analysis revealed 440 prokaryote and 139 fungal taxa significantly associated with pristine grassland, and 401 prokaryote and 168 fungal taxa associated with arable land use. A full list of these indicator taxa is provided in the Supplementary Materials, whereas dominant taxa are shown in (Figure 2.5).

Strikingly, the seven most abundant prokaryotic taxa indicative of Pristine grassland soils all belong to the phylum Verrucomicrobia (genera: Candidatus *Xiphinematobacter* and *DA101*), with other notable taxa occurring in the top 20 abundance-ranked indicators, including several α-Proteobacteria (genus: Bradyrhizobia, Rhodoplanes and Mesorhizobium) and Actinobacteria (genus: Gaiellaceae, Solirubrobacterales and Mycobacteriaceae). Prokaryotic indicators were abundant in arable soils and highly dominated by archaeal Candidatus *Nitrososphaera* taxa, as well as several other acidobacterial (iii1-15), firmicute (Sporosarcina, Planococcacaea and Bacillales) and actinomycete phyla (Arthrobacter) (Figure 2.5 top). Another notable taxon in the top 20 most abundant Arable indicators included a Nitrosomonad (β-Proteobacteria).

Fungal communities were dominated by *Mortierella minutissima*, which was abundant in both land-use types but was a significant indicator of Arable soils, whereas

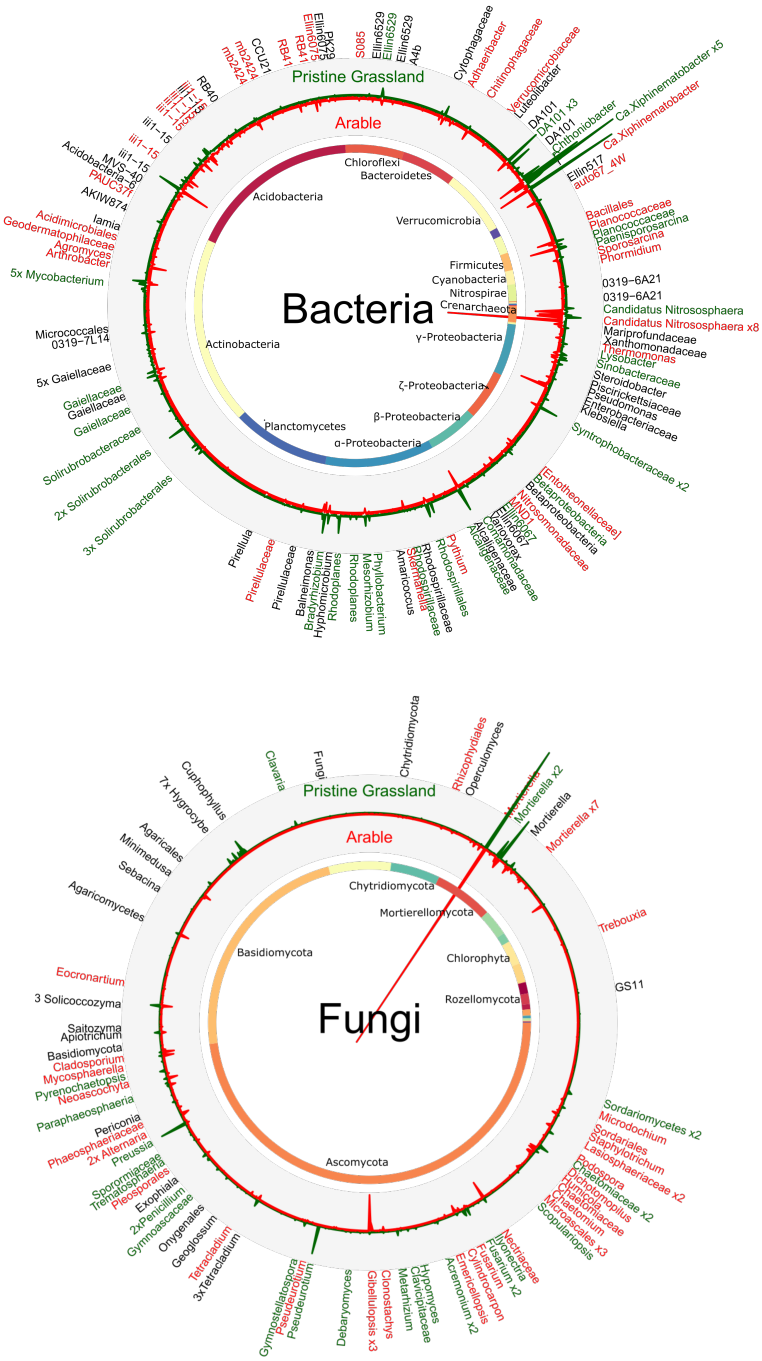


FIGURE 2.5: Circle diagram of (a) bacterial and (b) fungal indicators of grassland and arable soils. The mean relative abundance of 16S and ITS amplicons is plotted in red for Arable and green for Pristine grassland. Only dominant Operational Taxonomic Units (OTUs) are labelled, with red text denoting significant arable indicators, green denoting grassland indicators and black text identifying abundant taxa which are not affected by management

Mortierella exigua was dominant in Pristine grassland (Figure 2.5 bottom). Other abundant and significant fungal taxa in Pristine grassland soils were *Pseudeurotium, Preussia flanaganii, Fusarium solani* and *F. oxysporum* and *Clavaria*. Other dominant Arable soil indicators, aside from *Mortierella minutissima*, included *Gibellulopsis nigrescens*, *Cladosporium exasperatum*, *Mycosphaerella tassiana* and a member of the Nectriaceae family.

2.4.4 Indicator relationships with SOM restoration

In order to assess the performance of the arable and pristine grassland indicators in predicting SOM recovery with restoration management, we performed a pairwise Pearson correlation analyses of all microbial indicators and broader plant and microbial biodiversity metrics (diversity indices and ordination scores), together with soil abiotic and enzymatic responses. The correlation matrix is presented in Figure 2.6, displaying only those variables highly correlated with SOM (positive correlation in Figure 2.6 a, negative in Figure 2.6 b). SOM is positively correlated with the highly abundant Chthoniobacterales, an order of Verrucomicrobia, as well as with members of Rhizobiales and Syntrophobacterales.

The fungal OTU73 and Sordariales were also positively related, although they were found at lower relative abundance. As anticipated, there is a strong positive correlation of SOM with soil C, N, moisture and grassland age. In contrast, soil pH and C to N ratio are negatively correlated with organic matter and likewise with the highly abundant archaeal Nitrososphaerales, Actinomycetales, acidobacterial iii1-15 and RB41 taxa. We further visualize the specific relationships between SOM and the most dominant indicators of both land use and SOM restoration in Figure 2.7. The selected prokaryotic taxa *Nitrososphaera*, Ca. *Xiphinematobacter* and *Bradyrhizobium*, which were determined as indicative for Arable or Pristine land use, respectively, are more strongly correlated with SOM ($R^2 > 0.5$, p - value < 0.001) than the most abundant fungal specimen or extracellular acetase potential activity ($R^2 < 0.3$, p -value > 0.001) Figure 2.7.

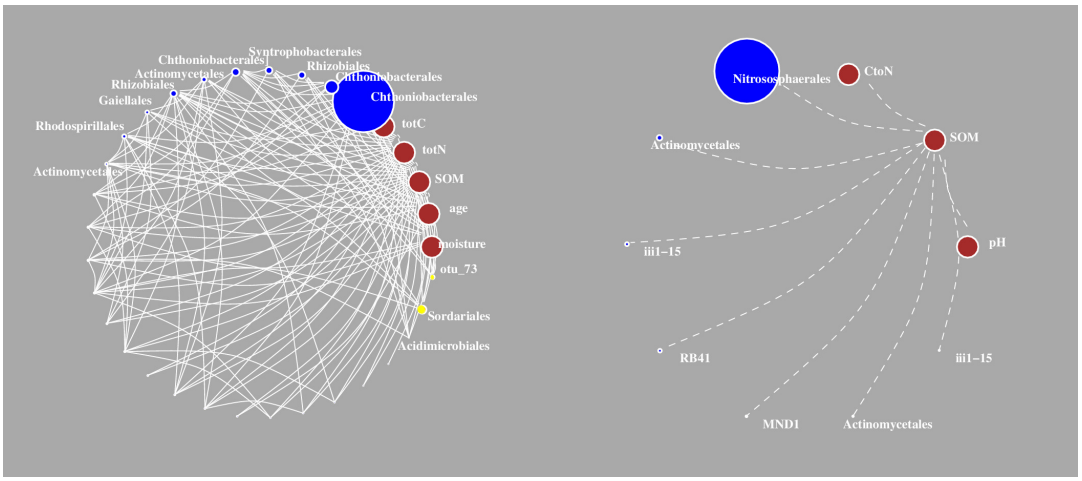


FIGURE 2.6: Network analysis of full dataset (soil chemistry, functional and biodiversity indicators) showing only strong correlations with SOM content. The left panel shows variables positively correlated with SOM (> 0.7) and the right panel shows negative correlations (< -0.7). For the molecular indicators the size of nodes is scaled to relative OTU abundance, and only the more abundant taxa are labelled. Blue nodes represent bacterial taxa, red nodes represent soil properties and yellow nodes are fungal taxa

2.5 Discussion

In this distributed survey of paired land-use contrasts, we found clear differences in plant, fungal and prokaryotic communities between historically undisturbed calcareous grassland soils and intensively managed arable land. Distinct bacterial, fungal and archaeal taxa were identified as highly indicative for each land use, and furthermore, a number of prokaryotic taxa were found to be the most strongly associated with grassland restoration age-related increases in SOM. The abundances of these specific taxa were found to be more sensitive indicators of SOM than any of the functional enzymatic responses or broader community metrics describing plant or microbial biodiversity.

Amongst the top bacterial indicators for pristine soils are several taxa of the phylum Verrucomicrobia. Our findings are consistent with previous studies which have demonstrated that members of the Verrucomicrobia are dominant across soils in different habitats and ecosystems (Bergmann et al., 2011), with a preference for grassland soils (Brewer et al., 2017). Our findings uniquely demonstrate that members of this phyla also strongly respond to increases in SOM brought about by grassland restoration. Although the lack of cultured representatives means we know little

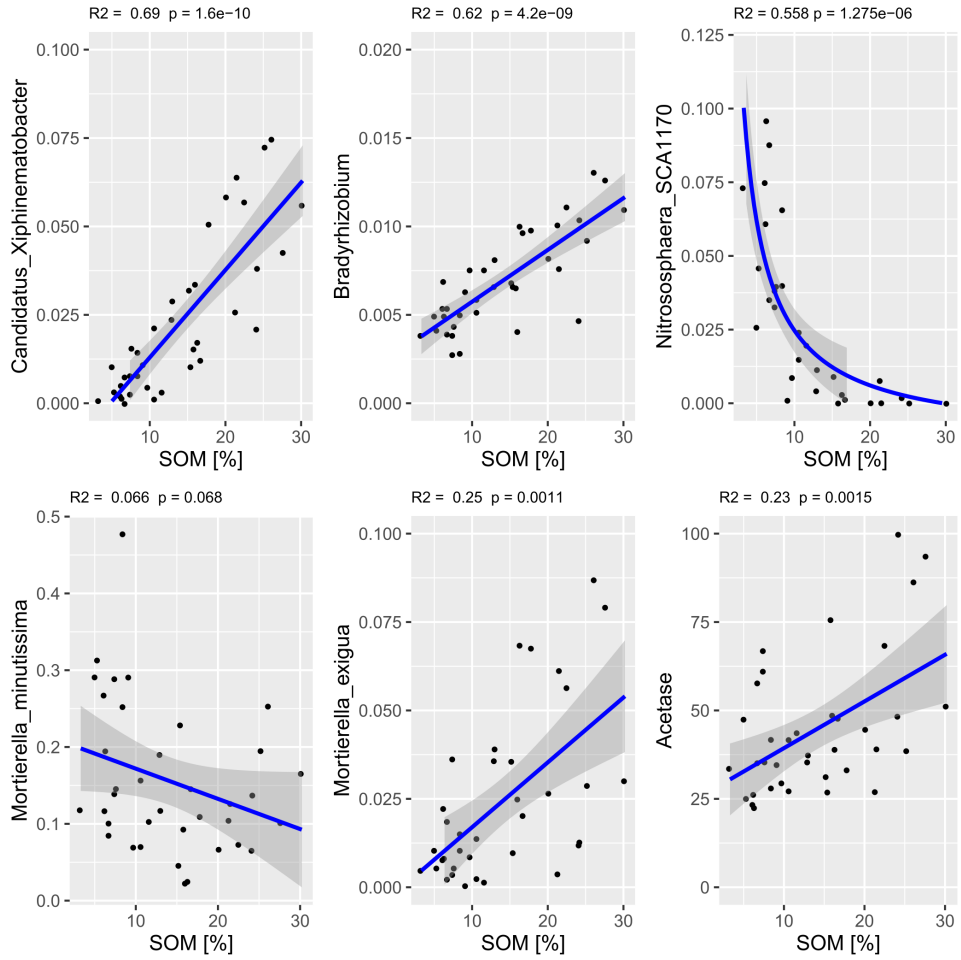


FIGURE 2.7: Top row: relative abundance of the three most dominant bacterial indicator taxa identified in the network analysis. Bottom row: other fungal and functional indicators were clearly related to SOM, but to a lesser extent than prokaryots. Ca. *Xiphinematobacter* and *Bradyrhizobia* are indicative for old grassland soils, whereas ammonia-oxidizing archaeal *Nitrososphaerales* indicate Arable land use. Grassland indicators increase in relative abundance with recovery of SOM in the restoration soils; *Nitrososphaerales* decrease. Acetase potential activities [nkat] and the abundance of indicator fungi *Mortierella exigua* are increasing with SOM, whereas *Mortierella minutissima* abundance decreases

about the functionality of Verrucomicrobia in soils, recent metagenomic reconstruction found evidence of heterotrophy with putative amino acid auxotrophies compensated by efficient mechanisms for amino acid uptake, and abilities to store surplus C (Brewer et al., 2017). Additionally, a reduced genome size was noted, which is thought to be a common phenomenon in free-living auxotrophic bacteria, which efficiently assimilate a wide range of compounds at low substrate concentration.

The arable soils were characterized by a dominance of several archaeal Nitrososphaerales taxa. Cultivated soils tend to contain elevated levels of nitrogen as a result of fertilizer application, which ammonia-oxidizers oxidize to nitrate in the first step of the nitrogen cycle (Boddy, 2016; Madigan et al., 2010). A functionally similar ammonia-oxidizing bacteria (AOB), a Nitrosomonad, was also found to be indicative of arable soils, but this was less abundant. AOB and ammonia-oxidising archaea (AOA), esp. Candidatus *Nitrososphaera*, were previously defined as signature organisms for agriculture in long-term experiments at one (Rothamsted Park Grass Experiment) or multiple locations and across a range of edaphic conditions (UK, Florida, Michigan), in which soil pH and ammonium concentrations were clearly correlated with AOA abundance. These studies also noted that the abundances of *Nitrososphaera* were negatively related to *Bradyrhizobium*, which was elevated in relatively unimproved plots (Zhalnina et al., 2013; Zhalnina et al., 2014). This is also consistent with our findings, as a bradyrhizobial taxon was also highly related to increases in organic matter, although less abundant overall than the Verrucomicrobia in these calcareous soils.

Previously, it was considered that the opposing abundances of these taxa in relation to N availability reflects differences in N capture, either archaeal ammonia oxidation in improved soils or bradyrhizobial N fixation in unimproved soils (Zhalnina et al., 2013). Although this may be true also in our soils, we also note that the recent metagenomics evidence suggests the Nitrososphaera are able to fix inorganic carbon from bicarbonate (HCO_3) or CO_2 (Berg et al., 2010), which also may be a factor underlying their competitiveness in C-depleted arable soils. Moreover, the slow-growing, free-living members of genus Bradyrhizobium were described to be

genetically highly heterogeneous, with certain taxa being unable to fix N in symbiosis with legumes, but different functions and carbon metabolisms depending on land use (Jones et al., 2016).

Although we found several fungal indicators of grassland versus arable management, when we included the restoration site data these did not respond as well as the bacterial indicators with respect to relationships with increasing SOM. *Mortierella*, a widely distributed soil fungus, was highly abundant across the soils and was also sensitive to land-use change. Although *Mortierella minutissima* dominated arable soils, *M. exigua* was found to be elevated in grassland soils. Previous studies on fungal communities under different land-management systems found *Mortierella* positively correlated to nitrate-N, but negatively to soil P (Detheridge et al., 2016), with *M. elongata* supporting crop performance by its contribution to the P cycle and increased activity of β-glucosidase and contributing to stable soil C pools via production of recalcitrant C compounds (Li et al., 2018).

We also found *Fusarium oxysporum* and*F. solani* as strong indicators for old calcareous grasslands and the potential plant pathogenic *Fusarium merismoides* as an indicator for arable land. Other potential plant pathogenic taxa from the classes Leotiomycetales and Dothideomycetales were amongst the top indicators for old grasslands (Sigler, Lumley, and Currah, 2000), confirming previous work showing uncertainties in the delineation between pathogenic and harmless saprotrophic fungi (Detheridge et al., 2016; Thornton, 1965). The investigated ITS marker gene targets identification of fungi, but picked up unicellular algae as indicative of croplands too, which are likely to form lichens and soil crusts. Using light as an energy source, they are able to grow on nutrient-deficient, bare surfaces (Watkinson, 2016).

More specific to croplands were a lichen, *Trebouxia decolorans*, and several green algae, as well as the crop pests *Alternaria infectoria* and *Stemphylium vesicarium*, the cause of spots on certain pears and a saprophyte in soil (Rossi et al., 2005). Neoascochyta species cause leaf scorch on wheat (Golzar et al., 2019) and were also more abundant in croplands. Interestingly, we detected the crop pathogen *Pythium* as an arable indicator when analysing the bacterial 16S sequencing output, where it came

up as a mitochondrial DNA sequence in the order α-Proteobacteria, which are ancestors of eukaryotic mitochondrial cells with their own genetic system (Bevan and Lang, 2004). As fungi are, like plants, spatially more variable than bacteria, their larger variance in soil molecular analysis is likely to be representative and reduces their potential as land-use indicators compared to the determined prokaryotic ones.

Extracellular enzyme activities in this study did not react consistently to land use, because responses within land-use classes were highly variable. Previous work has shown enzymatic responses can be highly affected by management, and in particular have been shown to be repressed with nutrient addition (Ramirez et al., 2014). However, in our study we have to consider not only the impact of fertilizer amendments, but tillage, pesticides, grazing and other plant growth stimulators, as well as the contrasting vegetation cover, which may have had unmeasured effects on the enzymatic responses. Other studies have also shown more variable responses across different enzymes across a chronosequence relating to specific nutrient limitations, but identified that correcting enzymatic responses to biomass better reflected efficiency in relation to successional changes in P acquisition (Allison et al., 2007). We also note that soil enzyme responses are known to be sensitive to temperature, season and assay pH (Nottingham et al., 2016; Puissant et al., 2019; Turner, 2010), factors we did not consider in our workflow of multiple substrate degradation assays from a single sampling point.

2.6 Conclusions

Soils provide fundamental services to humans and sustainable land management and restoration are crucial for maintaining soil multifunctionality in a changing world. Biological indicators are used widely for monitoring, although typical vegetation surveys are problematic because indicators may not be transferable between different sites and regions, due to differences in environmental factors (Karlík and

Poschlod, 2019). Additionally, the relevance of plant indicators for soil services remains uncertain. Our findings demonstrate that, across these calcareous soils, specific phylotypes of soil microbial taxa are the most consistent indicators of both land-use change and SOM recovery.

We therefore advocate that specific microbial taxa, and not broad taxonomic groups, be strongly considered amongst suites of indicators for soil monitoring (Bouchez et al., 2016; Griffiths et al., 2011). However, we note that our analysis was purposely limited to high pH soils, and so specific indicators for other geo-climatically defined soils remain to be defined.

More generally, the specific identification of microbial taxa responding to land-use change, and SOM improvement, should guide wider attempts to understand the functional capacity of these enigmatic organisms and their roles in driving soil formation and soil service delivery.

2.7 Supplementary Information

TABLE 2.3: Welch two-sided t-tests on soil state parameters comparing the land use categories Arable and Pristine grassland, n = 14.

	Arable	Pristine	t statistic	p-value	significance	lower CI	upper CI
SOM [%]	6.764286	22.16429	-10.2746	3.31E-08	***	-18.5931	-12.2069
Moisture [%]	20.82834	34.61232	-5.02493	0.000136	***	-19.6131	-7.95488
pH	7.930952	7.670238	3.52307	0.001619	**	0.108515	0.412914
P [g/kg]	13.95714	9.185714	1.901836	0.068417	.	-0.38761	9.930467
K [g/kg]	179.0643	145.6214	0.817753	0.422066		-51.2566	118.1423
Mg [g/kg]	48.12857	123.4786	-4.52604	0.000313	***	-110.529	-40.1708
Total N [g/kg]	0.358571	1.105714	-7.84139	1.05E-06	***	-0.95011	-0.54417
Total C [g/kg]	9.113571	13.55071	-3.97081	0.000514	***	-6.73551	-2.13877
C:N ratio	26.84286	12.73571	4.458271	0.000515	***	7.333283	20.881

TABLE 2.4: Welch two-sided t-tests on soil extra-cellular enzyme activities comparing the land use categories Arable and Pristine grassland, n = 14.

	Arable	Pristine	Arable - Pristine	t-statistic	p-value		lower CI	upper CI
Acetase	44.31	68.68	-24.38	-2.08	0.048	*	-48.57	-0.18
Phosphatase	13.71	12.45	1.25	0.45	0.656		-4.47	6.97
Chitinase	1.42	3.40	-1.98	-2.43	0.025	*	-3.69	-0.27
Hemicellulase	2.10	2.29	-0.19	-0.34	0.734		-1.35	0.97
Sulphatase	0.78	1.52	-0.74	-1.8	0.08		-1.58	0.11
Peptidase	9.97	16.98	-7.00	-2.10	0.051	**	-14.04	0.03
α-Glucosidase	2.38	1.27	1.11	1.87	0.080	.	-0.15	2.36
β-Glucosidase	14.38	12.80	1.59	0.51	0.618		-4.88	8.05

Chapter 3

Impacts of Conservation Farming on soil organic matter and microbial biodiversity indicators

3.1 Abstract

Conservation Agriculture promises to protect and restore soil health, which is otherwise negatively impacted by intensive farming practices. To date, there is a knowledge gap if changes from alternative management practices on soil microbial taxa are beneficial and how they relate to top soil organic matter recovery. In this chapter, a survey of 14 farm experiments distributed across the UK was conducted, which included different soil types and environments with trials, testing 35 varieties of cover crops, 9 reduced tillage practices and 2 organic amendments in combination with a variety of cash crops. SOM contents and soil bacterial, fungal and eukaryotic and indicator taxa were determined as a response to conservation agriculture and the relevant management types. At the broad level, SOM benefits from reduced tillage were stronger than from cover crops and compost. Soil microbial communities were mostly clustered according to Site, not by management. At one Site, SOM contents were 3% increased under CA after only 3 years of management change compared to intensive plough, and indicator taxa exclusively for this Farm were determined. Bacterial communities seemed more responsive to changes in OM than fungal or eukaryotic communities and delivered more indicator taxa than the other kingdoms.

The bacterial *Bradyrhizobium*, a previously described low intensity land use indicator, was enriched with CA, but not all microbial indicators were consistent with the findings of Chapter 2, as *DA101* and *Ca. Xiphinematobacter* were enriched under intensive tillage. Under CA, a higher relative abundance of potential pathogens was detected (*Pythium* and *Plasmodiophora*), but also of plant growth promoting bacteria and fungi (taxa belonging to the Rhizobiales and Chitinophagaceae; *Metarhizum* and *Trichoderma*, respectively). This work therefore identifies that management which increases SOM contents also impacts on previously observed "beneficial microbes" but more controlled, distributed experiments are needed to generalise across soil systems.

3.2 Introduction

It is widely recognised that intensive farming to produce food crops has harmful impacts on soil health, principally due to the detrimental impact of ploughing on soil organic matter stocks in upper soil layers (Tsiafouli et al., 2015; Lal, 2004b). Conventional cropping practices also often involve applications of industrially produced chemicals such as inorganic fertilisers and pesticides, which can lead to pollution of water bodies, and impact on soil biodiversity and wider soil ecosystem functions such as nutrient cycles and greenhouse gas emissions (Tsiafouli et al., 2015; Pierzynski et al., 2017; Sala et al., 2000). In order to feed growing human populations while minimising detrimental impacts on soil ecosystem services, it is widely recognised that new farming approaches are urgently needed which can maintain or even enhance productivity while minimising environmental impacts (Lal, 2019; Tilman et al., 2011; Foley et al., 2005). Indeed it has recently been proposed by the Food and Agriculture Organisation of the United Nations, that sustainable soil management requires a minimised level of erosion by maintaining a constant plant cover, enhanced SOM contents, balanced nutrient cycles and preservation and enhancement of soil biodiversity.

A number of specific management recommendations have been proposed to mitigate impacts of cropping on soil health including the use of in-field rotations, intercropping, field margins and hedges. More generally, crop management should strive to optimise soil organic matter (SOM) levels to support biodiversity, and pesticide and fertiliser applications should be minimised. A number of "conservation agriculture" (CA) approaches have therefore been proposed including reduced tillage ("min till"), cover cropping (CC), and application of organic material like compost or farm yard manure (FYM).

The claimed benefits of such approaches include the minimising of soil physical disturbance (min till), ecological soil nutrient enrichment and erosion limitation (cover crops), and building of soil organic matter through organic amendments which benefits carbon storage and moisture retention. With assumed benefits to soil biodiversity arising from maintaining diverse plant cover or organic amendments, it is predicted that the need for synthetic pesticide or nutrient additions can also be minimised through natural biological interactions (Vincent-Caboud et al., 2017). It has also been speculated that more ecological management can even improve crop quality with respect to plant constituents and nutrients (Rembiałkowska, 2007; Alyson E. Mitchell et al., 2007).

With respect to the scientific efficacy of these approaches, a number of long term minimum tillage trials have been performed globally, and the benefits to topsoil carbon improvement are reasonably well established, though uncertainties still surround net benefits with respect to soil C gain across the entire soil profile (Bongiorno et al., 2019; Prechsl et al., 2017; Powlson et al., 2014; Powlson et al., 2012). It is thought that reduced ploughing prevents physical mixing of topsoil carbon to deeper layers, but also prevents accelerated microbial decay of organic matter (Powlson et al., 2012; Powlson et al., 2014).

Cover cropping approaches (applied in combination with min till) are touted to also increase soil organic carbon stocks, especially in the top soil layers and help thus mitigating climate change (Lal, 2004a; Olson, Ebelhar, and Lang, 2014). Despite widespread adoption from industry, the wider benefits of cover crops, particularly with respect to plant variety used, has received comparatively less critical scientific

evaluation (Rillig and Lehmann, 2019; Wayman et al., 2014). It is thought that there is much scope for optimising plant traits to maximise soil benefits. For example, deep rooting plant species, e.g. radish, can improve soil structure and channel fresh plant carbon into deeper layers (Williams and Weil, 2004), whereas legumes are used to fix atmospheric nitrogen and reduce fertiliser requirements (McGuire, Bryant, and Denison, 1998; Kuo and Sainju, 1998). More generally, a higher plant biodiversity on croplands may benefit the local fauna including pollinators and soil invertebrates, as well as microorganisms, which may in turn be beneficial to the crop and maintain soil health.

Soil microorganisms fundamentally regulate many soil ecosystem functions, playing key roles in the cycling of carbon and nitrogen of relevance to crop productivity; as well as regulating the retention and losses of soil inputs of relevance to global biogeochemical cycles. Furthermore, they can directly interact with plants to either promote or reduce crop performance. Many plants benefit from plant growth promoting bacteria (PGPB) and symbiosis with fungi which benefit plant nutrient acquisition (Powell and Rillig, 2018; Detheridge et al., 2016). Microbial benefits including pathogen suppression, phytohormone production and nutrient solubilising compound secretion can act at all stages of plant growth, from germination through to the fruiting and senescence phase of a plant (Glick, 1995; Glick, 2010; Glick, 2014). Conversely, microbes can act as crop pathogens, causing significant economic impacts through diseases such as take all decline and Fusarium related vascular wilts.

Compared with effects on soil carbon, the impacts of new farming systems on soil biodiversity has received comparatively less attention, though this has been changing recently with the development of molecular methodologies to better characterise change in diverse soil communities. Intensive agriculture is known to be a key driver of biodiversity loss above- and below ground (Tsiafouli et al., 2015; Prechsl et al., 2017).

Intensively farmed systems, as well as being depleted in SOM, also typically exhibit decreased plant and animal richness. Soil faunal richness as well as microbial activity are also known to be reduced under intensive management (Lehmann et al., 2020; Chase et al., 2020), yet molecular estimates of microbial diversity actually appear to

show diversity increases (George et al., 2019), though this has been speculated to be due to issues surrounding DNA detection methodologies (Griffiths et al., 2016c). Irrespective of this, both local and large scale studies using molecular methodologies show intensive agriculture significantly changes soil microbes compared with unintensified contrasts (Armbruster et al., 2020; French, Tkacz, and Turnbull, 2017; Griffiths et al., 2016c). Whether new farming practices can ameliorate negative effects of intensive cropping practices on soil microbes remains a key question, which if true could add further political and economic incentives stimulating their adoption, beyond the benefits to topsoil carbon.

Key knowledge gaps remain with respect to our understanding of the effects on new farming practices on soil microbial communities. Firstly, despite recent advances in high throughput sequencing which provide semi-quantitative estimate of microbial taxon relative abundances, there is still a limited evidence base demonstrating impacts across a range of spatial scales (Zhalnina et al., 2013; Penton et al., 2014; Detheridge et al., 2016). Since soils can vary substantially due to natural differences in parent material and climate, it is possible that impacts of different managements will have differential effects across geo-climatic regions.

In concert, the management type and duration of application is heavily locally driven by farmer decisions, which in the absence of long term distributed experiments, adds further complications with respect to synthesis. More generally, there are large uncertainties with respect to understanding and predicting the functional consequences of altered biodiversity caused by different management decisions. Whilst for larger soil organisms, experimental reductions in biodiversity have been shown to reduce ecosystem functions (Bender, Wagg, and Heijden, 2016), manipulations of soil microbes have shown considerable resilience, possibly due to functional redundancy (Griffiths et al., 2001; Griffiths et al., 2004; Bell et al., 2005; Koziol and James D. Bever, 2016). To promote a case for adopting new management, for example to farmers, will likely require explicit linking of microbial change to specific functional benefits, rather than reporting on broad change in community diversity.

A better understanding of the wide scale effects of new farming practices on the soil microbiome could offer a number of benefits with respect to achieving sustainable

intensification. Firstly, with respect to innovating new management approaches, if it is possible to identify certain taxa causal in plant or soil related benefits then this opens the door to targeted attempts to manipulate the community. In this regard, the plant-soil microbiome is considered a potential tool for sustainable intensified agriculture, when successfully manipulated or maintained (Bender, Wagg, and Heijden, 2016; Coyle et al., 2016). This concept of ecological engineering could potentially be achieved via inoculation (Wubs et al., 2016), soil organic matter enhancement or smart crop rotations (Lehmann et al., 2020). Since plants attract and select a characteristic soil microbiome, better understanding of plant microbe interactions may inform this "smart agriculture", (Hu et al., 2018; Hartman et al., 2018; Hartman et al., 2017) particularly with respect to cover cropping. However, due to spatial considerations, intensive testing of a multitude of soil types and environments would likely be required, to specify the most suitable combinations of plant, tillage and nutrient addition for different sites (Rillig and Lehmann, 2019). Aside from innovating new management approaches, assessing the soil microbiome may also be useful to monitor the efficacy of new management practices. Since soil carbon can take a number of years to develop, it is possible that specific microbial taxa may serve as early indicators of beneficial processes in monitoring. Again, due to the heterogeneous nature of soils and uncertainties over redundancy, it is imperative that indicators are specified to local soil conditions, and are functionally relevant.

This chapter explores the impact of conservation agriculture (CA) on soil microbial communities and topsoil organic matter across a range of distributed sites in the South of England. I instigated this work as part of wider ongoing projects at CEH (NERC funded UGRASS and ASSIST projects), which had components seeking to engage farmers in research assessing the impacts of management options on soil health. In this survey of working farms I covered many different agricultural systems in realistic settings, to investigate the effectiveness of CA in restoring soil C and enhancing biodiversity of bacteria, fungi and eukaryotes. I was particularly interested in deploying a "Citizen Science" approach to my research; as participation of the non-academic community delivers not only high sample numbers and large

data sets, but furthermore engages wider stakeholders in scientific issues and environmental concerns (Schröter et al., 2017; Silvertown, 2009). In this regard, interested land owners were approached and provided with sampling kits, with a request to sample soil cores from adjacent conservation agriculture vs. conventionally managed fields. In return they would receive data on soil properties assessed, and could also partake in a workshop on the research outcomes.

Firstly, I aimed to quantify management impacts on topsoil organic matter contents across distributed locations in Great Britain. Furthermore, I want to compare the impacts of new management practices with other site related factors in driving biodiversity responses. With CA in practice, I especially hypothesise an enrichment of organisms that were previously defined as indicators of low intense land use (Armbruster et al., 2020) and reduced pathogen abundance and try to identify if the change in any specific microbial taxa are associated with OM recovery. Here, I expect an increased read abundance of organisms in conventional agriculture, which are stress resistant in terms of physical disruption, xenobiotics (eg. pesticides) and droughts.

3.3 Materials and Methods

3.3.1 Study Farms and sample collection

To cover a wide range of landscapes and new farming methods, I asked British agronomists and landowners of farms with cover cropping, reduced tillage, organic amendments or combinations of the treatments, to send us soil samples in a sampling kit which was provided. Five coil cores (2 cm diameter, 13 cm length) per site and treatment were collected from winter 2016/17 until March 2017, stored at -20 and sieved (2 mm) prior to further analysis. With the samples, I asked for detailed cropping and management history, co-ordinates and a sample from a near-by reference field of conventional farming as a control treatment. This was supported by UKCEH collaborators involved in sustainable agricultural intensification, like the ASSIST project and farming research companies (agrii, NIAB).

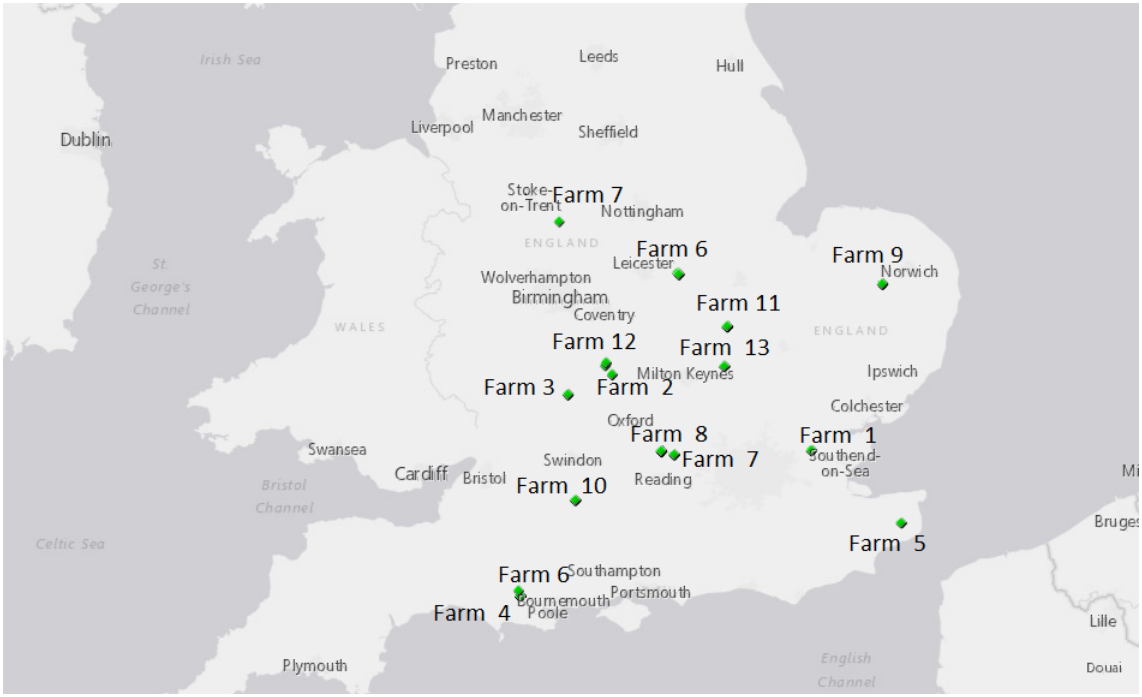


FIGURE 3.1: Location of Farms investigated for New Farming Systems. Sites differed in their experimental design from simple to nested treatments of reduced tillage, Cover Cropping with single and multiple plant species, different cash crops and rotations and addition of organic amendments from compost to chicken manure. The landscape scale furthermore included a range of soil and climate properties in the UK.

3.3.2 Soil Organic Matter contents

Organic matter content was measured as Loss-on-Ignition. Samples were oven dried at 105 ^oC until the weight did not change anymore to estimate soil moisture and further incinerated at 430 ^oC for 16 hours as recommended by NRM laboratories.

3.3.3 DNA sequencing of 16S, ITS and 18S amplicons

For DNA extraction, 0.15 g fresh soil was transferred into 96 well plates of the PowerSoil® DNA Isolation Kit (MO Bio Laboratories; Carlsbad; California). The sequencing was prepared according to (Kozich et al., 2013). In brief, amplicons were prepared using established primers for the ITS, 18S and 16S rRNA regions and protocols as summarised in Table 3.9.

For purification, amplicons were treated according to Zymo DNA Clean up Kit.

In a second round of PCR, Illumina adapters were added and all samples normalized using the SequalPrep ™ Normalization Kit (Invitrogen), pooled and concentration verified with Qubit. Illumina high throughput sequencing was performed with MiSeq® Reagent Kit v3 capable of producing 2 x 300 bp paired-end reads. For detailed workflow, follow instructions of github.com/SchlossLab/.

3.3.4 Bioinformatics and Statistical Analysis

Illumina sequencing output was analysed with the DADA2 package in R (R version 3.6.0, (Callahan et al., 2016)) to demultiplex raw sequences and trim paired sequences to uniform lengths. The core sequence-variant inference algorithm was applied with the DADA function to dereplicated data before paired-end sequences were merged and chimeras were removed.

Taxonomic data were assigned from GreenGenes (DeSantis et al., 2006) for bacterial and UNITE (Koljalg et al., 2005) for fungal taxonomy. Each phylotype abundance table was rarefied to 5000 reads, to account for differences in sampling depth, before assessing β-diversity in non-metric multidimensional scaling ordinations and running Permutational Multivariate Analysis of Variance (PERMANOVA) with the functions in vegan (Oksanen, 2008).

Significant ($p < .05$) indicator phylotypes for specific management practices were determined using the indval routine in labdsv (Dufrene et al., 2011) and wider statistical analysis and visualization was performed in R version 3.6.0 using the packages ggplot2 (Hadley Wickham, 2016) and labdsv (Roberts, 2019).

Statistical Analysis

Pairwise analysis of the treatments "Cover Cropping", "tillage system" or "organic amendment" versus conventional farming/plough per site was conducted. Student's t-tests or ANOVA/ANOSIM (was performed for differences in SOM content, depending on experimental design. Microbial communities from different sites were analysed in R! package vegan for similarity NMDS (metaMDS function), alpha and beta-diversity after rarefaction to the minimum number of reads and normalisation

using the decostand() function. Land management effects on soil OM content, bacterial, fungal and eukaryotic community composition, based on NMDS-scores, were subsequently fitted to OM contents using the envfit function in package vegan (Oksanen, 2008).
Indicators of the relevant treatments were determined as in Chapter 2, using the indval and simper functions in labdsv.

Land management effects on soil OM content, bacterial, fungal and eukaryotic community composition, based on NMDS-scores, were subsequently analysed using linear mixed effects models (lme4 package in R; Bates et al., 2014, with Cover Crop and tillage type (and organic amendment, where necessary), as fixed effects and Site as the random effect.

3.4 Results and Discussion

3.4.1 Effects of Conservation Agriculture on SOM

We engaged the farming community with help of UKCEH collaborators (stakeholders in sustainable agriculture and agronomists), defining our need for a conventional versus CA contrast, ideally replicated and in a proximal contrast. Farmers were then asked how many sampling packs would be required, in case they had access to several such contrasts within the land under their ownership. However, the samples returned often comprised multiple samples spanning a range of different experimental treatments applied within their farms (for example variations in tillage regime, and cover crop species). We expected comparisons between **conservation agriculture: CC or min-till or FYM** vs. **conventional agriculture ("Control"): no CC or ploughing or no FYM additions**.

In total, management contrasts from 13 different geographic locations were returned. Whilst we anticipated controls from all samples would be conventional ploughing with no cover crops, this was not always the case. In some farms (Farm 3, 4, 8, 10, 5, 9 and 11) the control treatment was also min till; and for other farms the control treatment also included different types of physical cultivation methods such

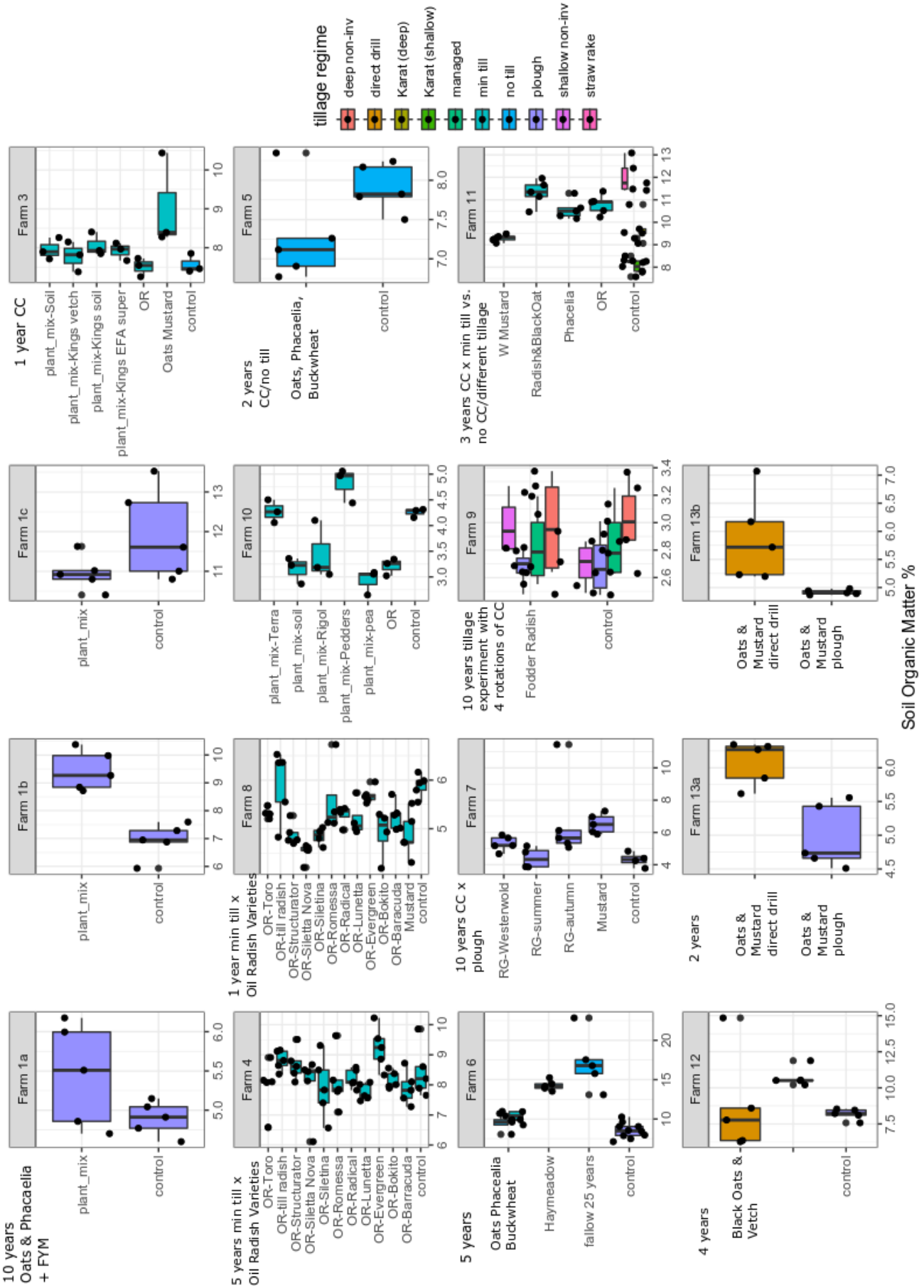


FIGURE 3.2: **Soil Organic Matter content [%] as response to reduced tillage, cover crops and organic amendments.** Soil Organic Matter content [%] as response to reduced tillage, cover crops and organic amendments in different crop rotations and soil types. Box-Whisker-plots display range of SOM content, black bar inside the box indicates mean. To the top left of each Farm is more detail on management, years of conservation agriculture on treatment was in place. Colours indicate tillage regimes.

TABLE 3.1: **Conservation agriculture effects on soil organic matter content across all investigated Farms (ANOVA)**. Soil organic matter was assessed as loss-on-ignition in [%]. Tillage and Cover cropping had significant effects on SOM contents, with tillage being a stronger driver than cropping.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
tillage	10	487.90	48.79	15.03	< 0.001
Cover Crop	36	1640.23	45.56	14.04	< 0.001
Cover Crop * tillage	6	27.39	4.56	1.41	0.2115
Residuals	319	1035.42	3.25		

as different tillage regimes (Farm 9 and 11).

With respect to conservation agriculture contrast, a number of different practices were returned, including CC, FYM either in combination or without minimum tillage. Additionally, six farms (Farm 3, 4, 7, 8, 10 and 11) also included a range of different cover crop species. Farm 4 and 8 tested different varieties of oil radish as CC.

Perhaps most importantly, the duration of implementation also differed from one CC rotation to 10 years (Figure 3.2).

In total, there were 38 different types of plant cover, including 12 commercially available plant mixes (Figure 3.2, Table 3.8). A hay meadow and a fallow field, which was not ploughed for 25 years were excluded from our analysis.

Two geographic locations were divided into sub-locations to account for the spatial differences within (Farm 1a -b- c and Farm 13a-b).

Overall, across the different farms SOM content was not significantly changed by CA (Welch Two Sample t-test comparing aggregates of [CC x min till/no till] (7.4 % SOM) vs. [noCC - plough] (6.5 % SOM), t = 1.8, p=0.07). The range of different treatments applied, and differing duration, meant a formal statistical analyses is inappropriate. We therefore discuss effects of CA management on SOM contents on a site by site basis.

Applying an ANOVA with the interaction of tillage and CC, the individual effects of CC and reduced tillage become more apparent (Table 3.1). Additionally, all treatments had been in place for different time points.

Together this means it is difficult to make conclusions on broad effects of ecological management. However, there were some sites which showed increases in OM

with minimal tillage and application of cover crops, within proximal contrasts. In particular, Farm 1, 4, 7, 9 and 11 were selected for DNA sequencing (Figure 3.2).

Short summary of Conservation Agriculture effects on SOM

Farm 3 - Short term trial reveals small benefits of CC compared with min-till + straw return

Farm 3 represented a one year trial of conservation farming. Contrasting control strips which were min tillage with return of crop straw residue only; with the treatment plots receiving one rotation of 6 different commercial CC mixtures, containing the following species: Kings EFA1 (oats and mustard); Kings EFA SUPER (Oil Radish, Black Oats, winter vetch, phacelia); Kings SOIL (2 types of radish with rye and oats); Kings vetch (Vetch and Rye); Soil Health Mix (rye, oats, phacelia, buckwheat, sunflower, peas, Oil Radish, Mustard, clover); and Oil Radish.

In a typical 3 year rotation the CC was sprayed off and residues left on the fields, before planting spring barley, oil seed rape, winter wheat, and the next CC. The mix EFA 1 with oats and mustard, resulted in the highest SOM content (7.57 +/- 0.24 in the control vs. 9.04 +/- 1.22 % in EFA1) and showed as well the highest variation of all tested treatments (Figure 3.2).

Farm 4 & Farm 8 - Oil Radish Variety trial as nitrogen catch crops

The trials at Farm 4 and Farm 8 were running for 5 years and 1 year, respectively and had similar experimental designs. The trial aimed to determine the CC variety that best reduces N leaching, a so called "Catch Crop" approach, to prevent water pollution in the Dorset catchment area.

A number of Oil Radish (OR) varieties were tested, which are marketed to improve soil structure and are hence recommended for reduced tillage management (product leaflet "Structurator" *Raphanus sativus*,
dlf.com/other-crops/catch-crops/species/other/chinese-radish/).

The deep root of this radish is claimed to break up compacted soil and enriches deep layers with more plant biomass than conventional oil radish varieties. The control strips were also managed with minimum tillage but had no CC. The experiment is laid out in duplicated strips of 8m width, each drilled at a constant seed rate of 15 kg/ha following Winter Wheat.

Nitrogen contents in soil and plant tissue were also monitored by the site owner but are not discussed further here. Comparison of the SOM contents at Farm 8 and Farm 4 show that the longer term trial had greater overall topsoil OM, possibly due to increased duration of conservation management, but could also reflect other differences in the natural SOM storage capacity across the two sites or differences in past management history. Moreover, only topsoil carbon contents were considered in the analysis and it is possible that deeper soil layers got enriched by deep rooting CC. Figure 3.2.

- Farm 4: 11 OR varieties (5 replicates per treatment), all min till, with WW stubble (8.44 % +- 0.866 SOM) as the "control". Strongest increase compared to the control was achieved with variety "Evergreen" (9.24 % SOM). The trial ran for 5 years and was implemented in the crop rotation with Winter Wheat and Winter Oats.

- Farm 8: 11 OR varieties and one plot White Mustard, same control treatment (5.89 % +-0.23). The trial incorporated one CC after a rotation of Spring Barley (2017), Spring Wheat (2016), Winter Oil Seed Rape (2015). Plots were rolled after direct drilling and the cover crop was terminated with glyphosate. In contrast to Farm 4, OR Evergreen had lower SOM than the control treatment and the other OR varieties did not enhance SOM contents. Highest SOM contents were under OR-Till Radish (5.93% +-0.7)

Folly Farm 5

At Farm 5, both the Cover Crop and the control plot were min-till. Cover Crop mixtures include soy beans, linseed, Phaecelia (planted in 2016) and winter vetch, Berseem Clover and Oil Radish in (2015). For only two years, cover cropping was

implemented in a rotation of cash crops with summer and winter wheat and summer barley. The SOM content at the cover crop site was slightly lower (7.28 +/- 0.63 % SOM vs. 7.9 +/- 0.3% in the control treatment), though it was not significant (t-test, p=0.093).

NIAB Farm 9

The nested experimental design included a Cover Crop (2 levels: with/without Fodder Radish) x 4 tillage treatments (levels: plough, deep non-inversive, shallow non-inversive and managed). An additional "managed" tillage treatment was also included based on measurements throughout the year and adapted farming practice, though we were unable to obtain specific details. A crop rotation of summer oats, winter wheat and oil seed rape without any organic amendments were maintained. Across all sites, Farm 9 had the lowest SOM overall. CC did not significantly increase SOM although the trial was running for 10 years. While SOM in the CC treatments did not differ from stubble controls (ANOVA F = 0.41, p = 0.528), significant effects from tillage regime were apparent (ANOVA F = 3.0677, p =0.0463 *).

Farm 7 Hurst Farm - mustard and rye grass cover for 9 years

At Farm 7, two Cover Crop species, mustard and rye grass (RG), were implemented in a rotation with maize. Maize was grown as cash crop at the site for 10 years and for 9 years, an additional crop was implemented every winter. Cow slurry was applied before drilling maize. Rye grass was investigated for the effect of variety (RG Westerwold vs. Italian RG) and sowing date ("RG-summer" = Italian RG planted in June together with RG Westerwold/ "RG-autumn" planted in October, together with Mustard "White Lightening"). The management included a half drill Cambridge Roll during spring. Rolling is a method to terminate the intercrop instead of spraying Glyphosate, but also flattens the surface, making it easier to seed at a constant depth and compacts the soil surface to maintain soil moisture, which is especially required during early stages of plant seedling emergence. All plots were ploughed. SOM

content was in general 1.5 % higher in soils with cover crops (Welch two sample t-test, p = 0.0025), but the effect size differed depending on plant type. For this site, mustard elevated SOM contents the most, while summer RG was the least effective.

Farm 6 Game and Wildlife Conservation Trust Loddington

At Farm 6, five years of cover cropping with a mixture of oat, buckwheat and phacelia were combined with min till or no till and compared to [no CC - plough]. A crop rotation with summer oats, winter wheat and oil seed rape was maintained. SOM contents under [CC - min till] were 10.4% (SD 0.56), [CC - no till] at 9.51 % (SD 0.97), whereas the conventional plough treatment without CC had 8.54% (SD 0.97) (Table 3.8). This field experiment was furthermore compared with a Haymeadow and a 25 year fallow field, which showed much higher SOM contents with 14.26 and 17.2 % respectively. These two subsites were not included in the analysis, as they did not meet the required assumptions.

Farm 11 Stow Longa Airfield

Farm 11 is laid out as an experiment in strips, including four plots with cover crops: phacelia, oil radish, white mustard and a mix of black oat/radish, which were all treated with minimum tillage. The experiment had run for three years when sampled. Another three plots without cover crop were subject to different tillage practices: Karat shallow, Karat deep and straw rake on natural fallow, as well as one conventionally ploughed treatment, which served as the "control" treatment in our analysis. Karat cultivators are adjustable in their tillage depth from 10 to 60 cm (depending on model), and can prepare the seed bed at the same time. Straw rake management means a simple raking of crop residues on the top soil layer. SOM content was highest in the fallow-straw rake soil and lowest in the ploughed treatment. CA significantly changed SOM (Welch t-test: t= 9.4, p < 0.0001), with 8.2 % in ploughed soil and 10.5 % in min till/CC plots.

3.4.2 Molecular analysis of microbial communities - effect of Site on soil biology

A subset of samples were selected to be sequenced in order to assess CA effects on soil microbial communities of the bacterial, fungal and protozoa kingdoms. Our choice was based on the increase in SOM contents and experimental duration and details in cropping and management history. Microbial communities were largely structured by Site (Figure 3.3). As the "baseline" SOM content at each location is different, the location wise clustering could be explained by OM. Bacterial communities were stronger related to OM (R^2 = 0.8) than eukaryotic and fungal communities (R^2 = 0.71 and 0.64, respectively). Still, there are broad consistencies across the microbial groups in their relation to Site. The first axis of the bacterial ordination is known to highly correlate with soil pH. Though not measured here, we cross referenced the ASVs evaluated here, with those from a larger GB wide survey (Jones et al., 2019a), and identified pH responsive indicator taxa. Examination of the responses of these across the first axis of the bacterial ordination revealed that the high pH sites are likely those to the left of the ordination (eg. Farm 4 and 11 around pH 7), and sites to the right being more acidic (with certain Farm 9 samples likely to be below pH 6).

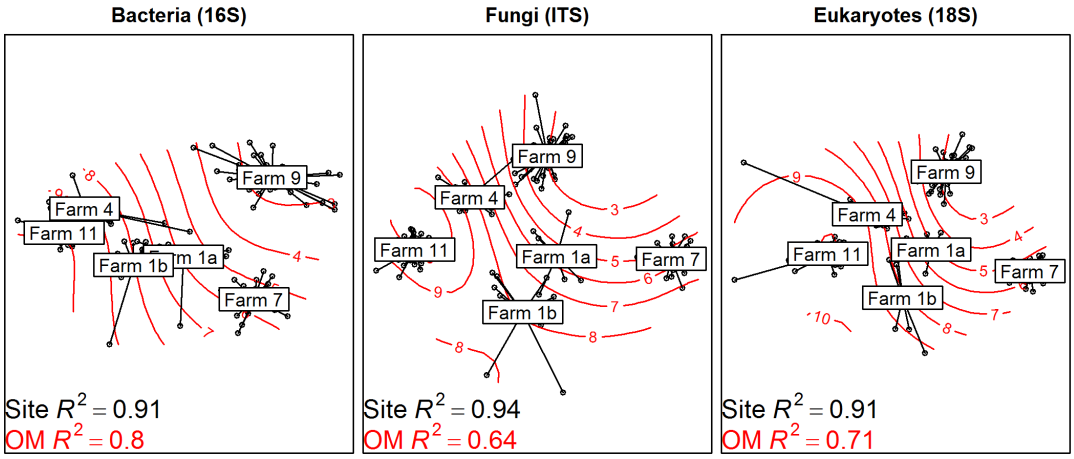


FIGURE 3.3: **NMDS ordination plots of bacterial, fungal and eukaryotic communities.** Microbial communities are largely structured by Site. Spiders connect sample points by site, with labels denoting farm name. Contours show nonlinear fits of soil organic matter (OM) to ordination site scores (ordisurf function in vegan). Inset within each plot are the effects of "Site" and "OM" as determined by linear fitting of both ordination axis to "Site" centroids and OM content (envfit function in vegan). Bacterial communities have the strongest linear relationship with soil organic matter.

TABLE 3.2: **PERMANOVA soil bacterial community composition.** PERMANOVA soil bacterial community composition as a function of location, Cover Cropping, tillage and addition of organic amendments (compost or farm yard manure)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Site	6	17.24	2.87	18.53	0.44	0.001	***
tillage	7	1.73	0.25	1.60	0.04	0.001	***
Cover Crop	1	0.38	0.38	2.43	0.01	0.012	*
amendment	1	0.35	0.35	2.25	0.01	0.012	*
Residuals	126	19.54	0.16		0.50		
Total	141	39.23			1.00		

3.4.3 Molecular analysis of microbial communities - effects of management

Strongest management effects on bacterial communities were detected at Farm 11 which includes three years experimental management, with a variety of CC and tillage approaches contrasted with standard ploughing (Figure 3.4).

Strong effects were also observed at Farm 1, which encompassed two sites differing in SOM, but was subject to conventional ploughing in both treatments. Notably, this

farm also had FYM added in the CC plots, so it cannot be ascertained if the change in microbial communities is from CC or the FYM addition.

Weak but significant effects of management became apparent at Farm 7, which had conventional ploughing with CC for 10 years. No significant effects were observed for Farm 4 which purely assessed impact of various CC under same min-till – possibly because this trial only ran for 1-2 years.

Farm 9, though non-significant, showed some evidence of clustering between conventional ploughing versus minimum tillage, irrespective of cover crop addition.

In total, the effects on bacterial communities largely mirror the OM effects, with strong effects mainly being apparent with altered tillage approaches rather than just cover crops.

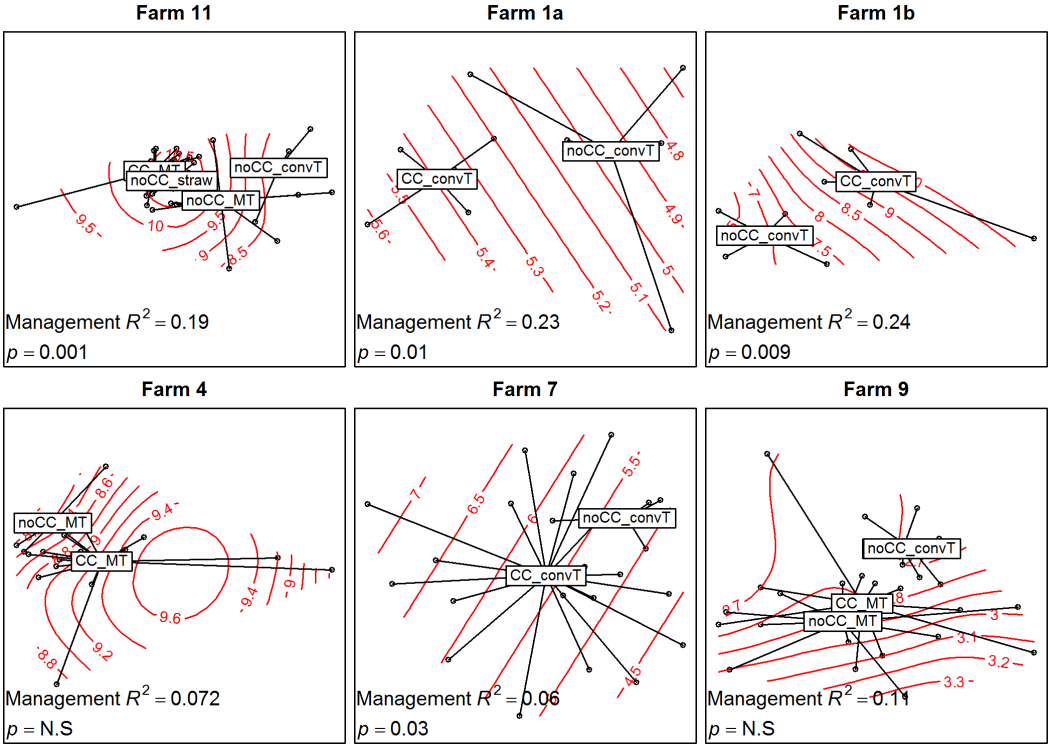


FIGURE 3.4: **NMDS ordination plots of Conservation Agriculture effects on soil bacterial communities.** Spiders connect sample points by broad CA treatment classifications, though specific managements differ across sites. Contours show nonlinear fits of soil organic matter (OM) to ordination site scores (ordisurf function in vegan). Inset within each plot are the effects of management as determined by permutational multivariate ANOVA (adonis function in vegan).

3.4.4 Molecular microbial indicators of change. A case study at Farm 11.

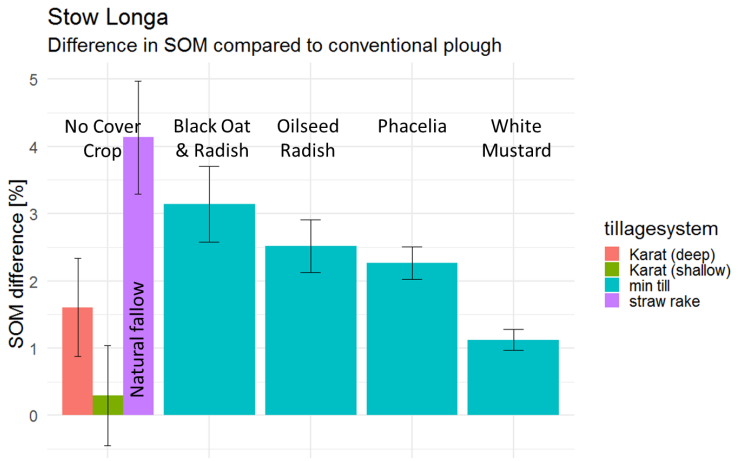


FIGURE 3.5: **Farm 11. Soil organic matter content increase** [%]. The plot without cover crop, that was conventionally ploughed served as control to four different cover crop treatments which were all managed with minimum tillage. Another three treatments at the Site without cover cropping showed increase in SOM stocks, but to a lesser extent. The "natural fallow" was manged with straw incorporation and had the highest SOM stocks.

Farm 11 is laid out as an experiment with included localised strip design:

four plots with the cover crops phacelia, oil radish, white mustard and a mix of black oat/radish, which were all treated with minimum tillage. The experiment ran for three years when sampled. The stakeholder, a well-known agronomy company, was particularly cooperative and interested in the results, so we organised a meeting to discuss the results. Primarily, the distinct separation of bacterial communities under different managements enthused me to further focus on this particular site and determine microbial indicators of conservation agriculture.

Another three plots without cover crop were subject to different tillage practices: Karat shallow, Karat deep and straw rake on natural fallow, as well as one conventionally ploughed treatment, which served as the "control" treatment in our analysis. SOM content was highest in the fallow/straw rake soil and lowest in the ploughed treatment (Figure 3.5).

Bacterial community was significantly changed by tillage and cover crops and again, tillage had a stronger effect than the additional crop (PERMANOVA: CC * tillage $F_{tillage}$=4.7, R^2=0.16, p = 0.001, F_{CC}=1.6, R^2=0.16,p = 0.004), (Table 3.3).

Indicator analysis was performed on the ploughed "control" vs. combined results of the four CC/reduced tillage strips, disregarding the fallow and Karat tillage plots. Indicator analysis was performed on the ploughed "control" vs. combined results of the four CC/reduced tillage strips, disregarding the no CC-reduced tillage plots. CA significantly changed SOM (Welch t-test: t= 9.4, p < 0.0001), with 8.2 % in ploughed soil and 10.5 % in min till/CC plots.

The number of significant indicator ASVs for conservation agriculture (minimum tillage and cover cropping) and intensive management (plough) at Farm 11, was not equally distributed among bacteria (16S rRNA amplicon) and eukarya/fungi (18S rRNA and ITS) (Table 3.4). Instead, there were multiple times more 16S indicators for both management practices than there were eukaryotic/fungal ones. This might be caused by a later development of the higher trophic levels, composed of nematodes, protozoa and arthropods, in soils after disturbance events (like intensive tillage) compared to bacterial and archaeal organisms. Moreover, prokaryotic organisms are more ubiquitous than those detected via ITS or 18S amplicon sequencing, with greater numbers of the same bacterial phylotypes found across the different treatments.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
tillage	1	0.52	0.52	4.73	0.16	0.001
Cover Crop	3	0.51	0.17	1.56	0.16	0.005
Residuals	20	2.20	0.11		0.68	
Total	24	3.23			1.00	

TABLE 3.3: **PERMANOVA Farm 11.**Bacterial soil community composition as a response to cover crop and minimum tillage. Comparison of plough/no cover crop vs. 4 different CC applications combined with min till.

TABLE 3.4: **Number of significant indicator ASVs for conservation agriculture (minimum tillage and cover cropping) and intensive management (plough)** in Farm 11, as case study presenting the indicator distribution among bacteria (16S rRNA amplicon) and eukarya/fungi (18S rRNA and ITS). Significant indicators are defined as indval p-values <0.05.

	conservation agriculture	intensive agriculture
bacterial 16S rRNA	71	226
eukaryotic 18S rRNA	13	76
fungal ITS	14	51

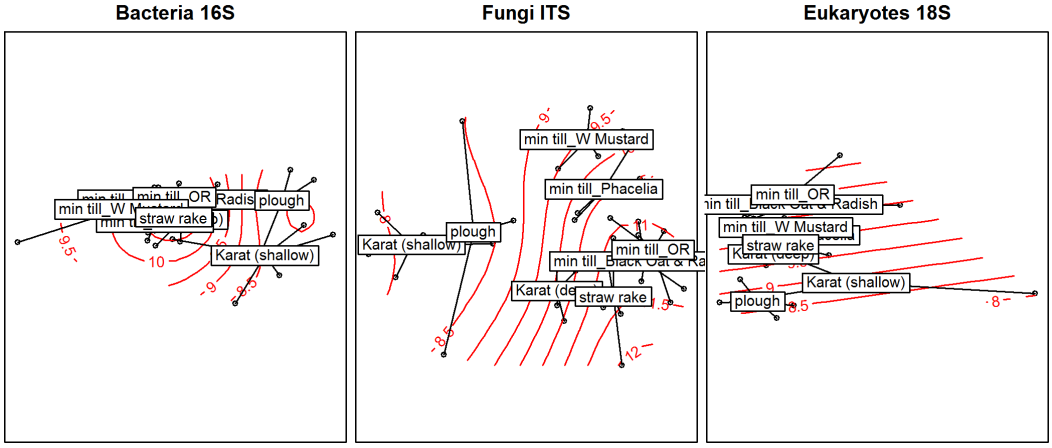


FIGURE 3.6: **NMDS ordination plots of Conservation Agriculture effects on soil microbial communities at Farm 11.** Conventional tillage (plough) and shallow tillage (Karat shallow) have distinct microbial communities corresponding with lowest OM contents irrespective of microbial group. Spiders connect sample points by specific CA treatment classifications. Contours show nonlinear fits of soil organic matter (OM) to ordination site scores (ordisurf function in vegan). Inset within each plot are the effects of management as determined by permutational multivariate anova (adonis function in vegan).

Bacterial indicators

The most abundant min till indicators were members of the acidobacterial order iii1-15, which were previously defined as indicators of pristine land use (Chapter 2). They highly correlated with SOM (R^2 0.4 and 0.6).

A rhizobacterial Bradyrhizobium of the class α -Proteobacteria, was defined as indicator of conservation agriculture, which was also previously found to be indicative of pristine land use, too. Another three CA indicators of the α -Proteobacteria were a member of genus *Kaistobacter*, and one of the genus *Skermanella*. A verrucomicrobial *auto67_4W OTU* was also indicative of min till and only weakly associated with SOM.

In contrast to this and the findings from Armbruster et al., 2020, verrucomicrobial *Candidatus Xiphinematobacter* and DA101 were found to be indicative of intensive land use in this specific case. Interestingly, they were inversely related to OM contents, with R^2 = -0.2, -0.3 and -0.5 respectively (Table 3.5).

In ploughed soil, the most abundant significant bacterial indicators were OTUs from the family Gaiellaceae and the order 0319-7L14 (both Phylum Actinobacteria), Saprospiraceae (Phylum Bacteroidetes), three Bacillus OTUs, one *E. coli* and the three above mentioned verrucomicrobial taxa.

Gaiellaceae were described as strictly aerobic, chemoorganotrophic and able to reduce nitrate, features well suited for soils aerated by intensive tillage and fertilizer caused high nitrogen contents (Rosenberg et al., 2014). It can be speculated that the verrucomicrobial OTUs are enriched in the plots with lower organic matter stocks, which show thus more alkaline soil pH.

TABLE 3.5: **Bacterial indicators of conservation agriculture.** Bacterial most abundant, significant indicator ASVs for conventional plough vs. minimum tillage at Farm 11. Significance refers to p - value <0.05 of indval indicator value (indval analysis). Abundance refers to mean abundance of 16S amplicon DNA sequencing reads. R^2 SOM ...linear relationship between Soil Organic Matter contents and indicator mean abundance in the field experiment.

Bacteria	**min till**					
Phylum	Class	Order	Family	Genus	Species	R^2 SOM
Acidobacteria	Acidobacteria - 6	iii1-15				0.51
		iii1-15				0.37
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae			0.5
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae			0.14
Proteobacteria	α -Proteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*		0.45
		Rhodospirillales	Rhodospirillaceae	*Skermanella*		0.34
		Sphingomodales	Sphingomodaceae	*Kaistobacter*		0.47
	β -Proteobacteria	Nitrosomodales	Nitrosomodaceae	*Nitrosovibrio*	*tenuis*	0.3
	γ -Proteobacteria	Xanthomodales	Sinobacteraceae			0.33
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	auto67_4W			0.13
	Intensive till					
Actinobacteria	MB-A2-108	0319-7L14				-0.16
	Thermoleophilia	Gaiellales	Gaiellaceae			-0.55
Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae			-0.57
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus		-0.61
				Bacillus		-0.66
				Bacillus		-0.48
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia*	*coli*	-0.55
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	*Candidatus Xiphinematobacter*		-0.20
				DA101		-0.37
				DA101		-0.49

Fungal and other eukaryotic indicators

Globally, fungal soil communities are dominated by the wind-dispersed phylum Ascomycota and the phylum Basidiomycota (Egidi et al., 2019). From the phylum Ascomycota, I found *Exophiala equi*, *Clonostachys rosea*, one member of the family Nectriacea and *Chaetomium funicola* to be enriched in their read abundance under CA management. *Trichoderma* spp. are commercially applied agents in agriculture, because this free-living fungus secretes a wide range of antibiotic compounds and is highly interactive in soil and litter environments. It can induce systemic resistance in plants against pathogenic fungi and insects and its spores are therefore applied to field soil to protect crops from parasitism (Harman et al., 2004). The endomopathogenic fungus *Clonostachys rosea* can support plant performance by reducing the stress caused by other pathogenic fungi (Xue, 2007) and showed the second strongest correlation with SOM (R^2 = 0.46). Two members of the Basidiomycota were furthermore indicative for CA treatment as assessed with the 18S amplicon sequencing, one OTU of the order Agaricales.

Metarhizium marquandii, previously described and categorized as the synonym *Paecilomyces marquandii*, is touted as plant protective insecticide and growth stimulating in maize, beans and soy by secretion of indole acetic acid (IAA) and increased soil P availability (Baron, Souza Pollo, and Rigobelo, 2020). This fungus (phylum Ascomycota) had the highest positive correlation with SOM of R^2 = 0.5 in the indicators determined by ITS amplicon sequencing.

A higher relative abundance of fungal *Pythium* DNA was found to be indicative of CA, a plant parasitic genus for many crops, which includes non-pathogenic species, too. In the case of *Fusarium*, there is evidence that some members of this genus are not harmful to the plant, but protect it instead from attacks of vascular wilt causing *Pythium ultimum* (Harman et al., 2004). Several *Pythium* species cause vascular wilt in a very broad host range and thus immense economic damage to the farming industry. However, not every species of this fungus is pathogenic under the given environmental parameters and there is a now technological potential to evaluate the metabolic toolbox with whole genome transcriptomics, investigating proteins targeting adhesion to plant membranes, and to functionally assess its growth on different

carbon sources, to understand the fungal pathogenicity strategies and plant defence mechanisms (Lévesque et al., 2010).

There was also an increase of the genus *Plasmodiophora* (phylum Cercozoa, Phytomyxea family) associated OTUs in min till soils, which was of particular interest for the agronomist stakeholder. *Plasmodiophora* is a plant parasite in the supergroup Rhizaria, which causes cabbage club root in Brassica crops, but was shown to be suppressed by endophytic fungi like *Acremonium* (Jäschke et al., 2010; Bulman et al., 2007). In this Farm 11 case study, its relative abundance correlated strongly with OM contents (R^2 = 0.4) Table 3.6 and 3.7.

Another cercozoan OTU of the class Glissomonadida was indicative for conservation agriculture, which was not further classified. The Glissomonadida were shown to dominate soil protist communities in temperate grasslands and include bacterivore and eukaryvore families, with a wide variety of morphological and motility traits (Fiore-Donno et al., 2019). Other significant and abundant min till eukaryotic indicators include one OTU of order Argaricales (Basidiomycota), an OTU of the family Chromuliles, and a member of the order Rhizophydiales (phylum Chytridiomycota), which were not further identified. In contrast to the CA indicators, the dominant fungal and eukaryotic taxa under intensive tillage were inversely correlated to SOM contents. Amongst the top most abundant indicators for intensive management were an OTU of the Pleosporales (Ascomycota), one member of the genus *Solicoccozyma* and another one of the class Agaricomycetes (both of the phylum Basidiomycota). The highest inverse correlation with SOM were found in *Solicoccozyma* (R^2= -0.67), *Ceratiomyxella* (R^2= -0.58) and Pleosporales OTU(R^2= -0.53).

Another intensive farming indicator was one OTU of the genus *Acanthamoeba* (Phylum Amoeboid, class Discosea, domain Eukaryota), which is commonly found in soil and water environments, that includes infectious species but it was not further identified as one of the harmful specimen. The two life cycle stages of these ameba can serve as reservoir of bacteria with human health damaging effects (Marciano-Cabral and Cabral, 2003). One OTU of the ubiquitous order Mortierellales (phylum Mucoromycota, (R^2= -0.45)) and *Ceratiomyxella* (Phylum Schizoplasmodiida), R^2=-0.58, commonly found on decaying litter (Schnittler et al., 2012) were also indicative

for intensive tillage. One member of the family Haptoria (Ciliophora) (R^2= -0.3).

Three *Mortierella* OTUs, *M. exigua*, *M. gamsii*, *M. alpi* were determined as indicative for intensive farming, though the abundance in undisturbed pristine grassland soils made *Mortierella exigua* an indicator for extensive land use in high pH soil systems (Chapter 2). Mortierella are commonly dominating fungal communities in soils and were shown to support plant growth by positively contributing to the phosphorus cycle. Thus, they are adapted to low P soils and their abundance correlates with nitrate nitrogen (Detheridge et al., 2016; Li et al., 2018). Two *Cryptococcus aerius* OTUs (phylum Basidiomycota, class Tremellomycetes), which are aerobic, amylase secreting fungi known from the wood industry, inhabiting soil and sand (Shafiee, Nahvi, and Emtiazi, 2005) and one of the genus Powellomyces were also intensive farming indicators.

In this specific comparison, the fungus *Olpidium brassicae* (phylum Olpidiomycota) was indicative for intensive tillage without CC, although no brassica crop was growing on the field. This potentially pathogenic fungus was previously described as indicator of arable land use when compared to soils with natural species-rich grassland cover (French, Tkacz, and Turnbull, 2017). One *Papiliotrema laurentii* OTU was found to be indicative for CA whereas another one indicated ploughed soil. Again, this points out the importance of a high taxonomic resolution when investigating soil microbiota.

TABLE 3.6: **Fungal indicators of conservation agriculture.** Fungal most abundant, significant indicator ASVs for conventional plough vs. minimum tillage at Farm 11. Significance refers to p - value <0.05 of indval indicator value (indval analysis). Abundance refers to mean abundance of ITS amplicon DNA sequencing reads. "R^2 SOM " ...linear relationship between Soil Organic Matter content and indicator mean abundance in the field experiment at Farm 11.

Fungi (ITS)	**min till**					
Phylum	Class	Order	Family	Genus	Species	R^2 SOM
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*equi*	0.32
	Leotiomycetes	Incertae sedis	Pseudeurotiaceae	*Pseudeurotium*		0.01
	Sordariomycetes	Hypocreales	Bionectriaceae	*Clonostachys*	*rosea*	0.46
			Clavicipitaceae	*Metarhizium*	*marquandii*	0.49
			Hypocreaceae	*Trichoderma*		0.24
			Nectriaceae			0.28
		Microascales				0.04
		Sordariales	Chaetomiaceae	*Chaetomium*	*funicola*	0.28
Basidiomycota	Microbotryomycetes	*Incertae sedis*	Chrysozymaceae			0.21
	Tremellomycetes	Tremellales	Rhynchogastremataceae	*Papiliotrema*	*laurentii*	0.36
	Intensive tillage					
Ascomycota	Leotiomycetes	Leotiomycetes *Incertae sedis*	Pseudeurotiaceae			-0.11
Basidiomycota	Agaricomycetes	Agaricales				-0.57
	Tremellomycetes	Tremellales	Rhynchogastremataceae	*Papiliotrema*	*laurentii*	-0.49
			Tremellaceae	*Cryptococcus*	*aerius*	-0.62
				Cryptococcus	*aerius*	-0.57
Chytridiomycota	Spizellomycetes	Spizellomycetales	Powellomycetaceae	*Powellomyces*		-0.45
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*exigua*	-0.36
Mortierellomycota				*Mortierella*	*alpi*	-0.54
Mortierellomycota				*Mortierella*	*gamsii*	-0.34
Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	*brassicae*	-0.57

TABLE 3.7: **Eukaryote indicators of conservation agriculture.** Eukaryote most abundant, significant indicator ASVs for conventional plough vs. minimum tillage at Farm 11. Significance refers to p - value <0.05 of indval indicator value (indval analysis). Abundance refers to mean abundance of 18S amplicon DNA sequencing reads. "R^2 SOM ...linear regression between Soil Organic Matter content and indicator mean relative abundance in the field experiment.

Eukaryota	**min till**					
Phylum	Class	Order	Family	Genus	Species	R^2 SOM
Basidiomycota	Agaricomycetes	Agaricales				0.40
—				*Saccamoeba*		0.22
Cercozoa	Glissomodida					0.16
Cercozoa	Phytomyxea	Phytomyxea	Phytomyxea	*Plasmodiophora*		0.42
Chytridiomycota	Incertae Sedis	Rhizophydiales				0.34
Cryptomycota						0.39
Ochrophyta	Chrysophyceae	Chromuliles	Chromuliles			0.12
Peronosporomycetes				*Pythium*		0.5
			uncultured eukaryote			0.1
			uncultured eukaryote			0.26
	Intensive tillage					
Ascomycota	Dothideomycetes	Pleosporales				-0.53
Ascomycota						-0.33
Basidiomycota	Agaricomycetes					-0.43
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*		-0.67
Cercozoa						-0.53
Chytridiomycota	*Incertae Sedis*	Spizellomycetales	Powellomycetaceae			-0.47
Ciliophora	Intramacronucleata	Litostomatea	Haptoria			-0.30
Discosea	Longamoebia	Centramoebida	Centramoebida	*Acanthamoeba*		-0.49
Mucoromycota	*Incertae Sedis*	Mortierellales				-0.45
Schizoplasmodiida				*Ceratiomyxella*		-0.58

3.5 Conclusions

In the farming context, there is a limited number of scientific studies examining the effects of conservation agriculture on soil health in Britain (Quinton and Catt, 2004; Ecclestone, 2001; Turley et al., 2003), and Europe (Putte et al., 2010). These farm experiments consider several types of soil, management, plant cover and ecological responses but not the effects on microbial diversity and keystone taxa. Hence, there is a need to synthesise whether the same microbial taxa respond to management at larger scale upon change in SOM stocks.

The sustainable agricultural methods investigated in this British farm survey incorporated 35 varieties of cover crops, 9 tillage managements, 2 types of organic amendment and a variety of cash crops and rotations across 14 sites. To my knowledge, this is the first survey of CA in a realistic agricultural setting in a range of different soil and farming systems in the UK.

However, this realistic setting was problematic for drawing general conclusions, due to the different managements, differences in environment and climatic conditions at the sites and the differing time points since when the new farming methods were put into practice. Because of this, it was difficult to generalise the findings across sites, with Site most strongly discriminating microbial community clusters in the national comparison, blurring the management effects on both, top soil organic matter contents and soil biodiversity responses. For this reason, I described one Site in detail in respect to microbial indicator organisms. In the case of example Farm 11, SOM contents under CA were increased by up to 3% after only 3 years management change, with the biggest improvements in reduced tillage practices (Figure 3.5).

An enrichment of bacterial taxa that were previously described as indicators of low intensity land use was given in the case of α-Proteobacteria, especially *Bradyrhizobium* but not all microbial taxa seemed to be consistent in their indication. Verrucomicrobial *Ca. Xiphinematobacter* and *DA101* for example, showed higher relative abundances in ploughed, intensive management and acidobacterial iii1-15 enriched in intensively managed, as opposed to the findings in Chapter 2.

It has to be considered, that the differences between natural, species-rich grassland and cropland soil (communities) is not comparable with variances between

two arable soils, albeit that one of the two is used less intensively than the other.

In terms of pathogen abundance, *Plasmodiophora*, (member of pathogen rich order of Phytomyxea) and *Pythium* were increased under CA. These results should be interpreted carefully. It is impossible to certainly determine a microbial taxon as crop pathogenic or beneficial by DNA metabarcoding only. Nevertheless, we found an increased relative abundance of known plant growth supporting fungi *(Metarhizum marquandii, Trichoderma*) and bacteria (Rhizobiales, *Chitinophagaceae*) under reduced tillage which protect and support plants according to scientific literature.

In summary, there were indications that tillage system was more important in affecting organic matter than CC *per se*, with greater gains in SOM at all six sites which had tillage change as experimental factor. Furthermore, there was evidence for effects of different CC varieties on both, OM and microbial communities. Site was found to be more important in affecting communities than specific farming practices. This emphasises the need for distributed, replicated field experiments with contrasting managements across different geographic locations.

Clearly, changes in microbial communities also occurred where there were large changes in OM contents. At broad level, bacterial communities seemed more responsive to changes in OM than fungal or eukaryotic communities and delivered more indicator taxa than the other kingdoms. In regard to indicator organisms of management intensity, I examined indicators specifically for one site as case study and discussed their functional relevance together with interest from stakeholders (AGRII). Informal examination across other sites did not reveal any obvious consistency in OM responsive indicators, which was not pursued given the large differences in site managements.

3.5.1 Recommendations

In order to make a transition to sustainable agriculture, soil quality and especially SOM has to be maintained and monitored. In order to evaluate the efficacy of new farming systems, there is now a need to develop indicators and assess their consistency by long term valid experimental comparisons. The findings of this survey

suggest to use bacterial indicators rather than fungal or eukaryotic one, as prokaryotic communities were more responsive to management and SOM changes.

Moreover, we need to validate how changes in indicators can assess function to enhance scientific understanding, develop new managements, monitor efficacy and develop means to communicate information that stakeholders want (eg. a specific management will enhance microbial taxa, which will increase productivity and benefit soil health etc).

In a whole day workshop with interested stakeholders of the agronomist company agrii, members of the UKCEH Biodiversity and Soils and Land Use Departments, I presented the findings and we discussed problems and future scenarios of sustainable soil management. For that purpose, a simple semi-quantitative scorecard with the most interesting microorganisms was developed, which was rated to be most useful for a non-scientific audience (see an example in Figure 3.7).

Future research investigating direct microbe-plant interactions needs to proof plant pathogenicity and benefits in experiments under controlled conditions with microbial pure cultures of the indicators, in order to fully understand their ecological function. Moreover, whole genome sequencing would provide insights into the life strategies and traits that are emerging with changes in land use transitions.

3.6 Supplementary Data Chapter 3

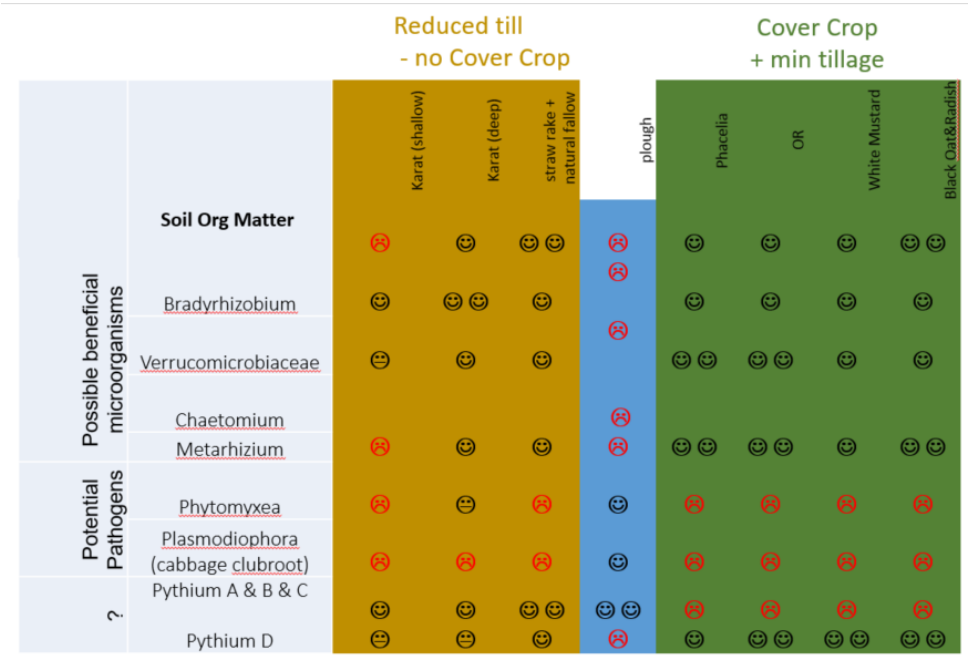


FIGURE 3.7: **Illustrative Example of an Indicator Scorecard for Conservation Agriculture**. This scorecard was developed to communicate molecular microbiology findings at the specific Site (Case Study Farm 11) to the farming community. Columns with specific management practices show the effect on relative abundances of indicator microorganisms, which are potentially of interest in cropping systems. Smiley faces: elevated abundances of beneficial, plant growth promoting organisms or decrease in crop pathogens; *vice versa* sad faces: elevated pathogen abundance and suppression of beneficial microbes. Here, intensive tillage (blue column) showed mostly sad faces.

TABLE 3.8: **Overview of mean Soil Organic Matter contents at the surveyed Sites implementing conservation agriculture.** Mean SOM contents determined as Loss-on-Ignition, grouped by Cover Crop mix or species and tillage system. "control" in the cover crop column refers to no Cover Crop was grown. FYM ... Farm Yard Manure, OR ... Oil Radish, RG ... rye grass, mix ... 2 or more cover crop plant species and /or varieties were established

Site	Cover Crop	tillage	organic a	SOM [%]	SD
Farm 1a		plough		4.89	0.22
Farm 1a	mix	plough	compost	5.45	0.66
Farm 1b		plough		6.93	0.63
Farm 1b	mix	plough	compost	9.44	0.72
Farm 1c		plough		11.93	1.16
Farm 1c	mix	plough	compost	10.95	0.44

Farm 3		no till		7.57	0.25
Farm 3	mix-Kings EFA super	min till		7.92	0.23
Farm 3	mix-Kings EFA1	min till		9.04	1.22
Farm 3	mix-Kings soil	min till		8.06	0.30
Farm 3	mix-Kings vetch	min till		7.78	0.39
Farm 3	mix-Soil	min till		7.95	0.28
Farm 3	Oil Radish	min till		7.51	0.24
Farm 4		min till		8.44	0.87
Farm 4	Oil Radish -Barracuda	min till		7.90	0.55
Farm 4	Oil Radish -Bokito	min till		8.16	0.29
Farm 4	Oil Radish -Evergreen	min till		9.24	0.71
Farm 4	Oil Radish -Lunetta	min till		7.76	0.28
Farm 4	Oil Radish -Radical	min till		8.21	0.32
Farm 4	Oil Radish -Romessa	min till		8.10	0.94
Farm 4	Oil Radish -Siletina	min till		7.92	1.04
Farm 4	Oil Radish -Siletta Nova	min till		7.97	1.06
Farm 4	Oil Radish -Structurator	min till		8.68	0.53
Farm 4	Oil Radish -Till radish	min till		8.82	0.33
Farm 4	Oil Radish -Toro	min till		7.97	0.84
Farm 5		no till		7.90	0.30
Farm 5	mix	no till		7.28	0.63
Farm 6		plough		8.54	0.97
Farm 6	mix	min till		10.40	0.56
Farm 6	mix	no till		9.51	0.97
Farm 7		plough	FYM	4.32	0.44
Farm 7	Mustard	plough	FYM	6.54	0.61
Farm 7	RG-autumn	plough	FYM	6.73	2.66
Farm 7	RG-summer	plough	FYM	4.42	0.65
Farm 7	RG-Westerwold	plough	FYM	5.31	0.46
Farm 8		min till	FYM	5.89	0.23
Farm 8	Mustard	min till	FYM	4.88	0.47

Farm 8	Oil Radish - Baracuda	min till	FYM	5.26	0.29
Farm 8	Oil Radish - Bokito	min till	FYM	4.91	0.51
Farm 8	Oil Radish - Evergreen	min till	FYM	5.69	0.17
Farm 8	Oil Radish - Lunetta	min till	FYM	5.20	0.37
Farm 8	Oil Radish - Radical	min till	FYM	5.28	0.21
Farm 8	Oil Radish - Romessa	min till	FYM	5.58	0.77
Farm 8	Oil Radish - Siletina	min till	FYM	4.85	0.18
Farm 8	Oil Radish - Siletta Nova	min till	FYM	4.52	0.18
Farm 8	Oil Radish - Structurator	min till	FYM	4.88	0.23
Farm 8	Oil Radish - Till Radish	min till	FYM	5.93	0.72
Farm 8	Oil Radish - Toro	min till	FYM	5.32	0.10
Farm 9		deep non-inv		3.06	0.24
Farm 9		managed		2.86	0.29
Farm 9		plough		2.70	0.24
Farm 9		shallow non-inv		2.70	0.17
Farm 9	Fodder Radish	deep non-inv		2.98	0.37
Farm 9	Fodder Radish	managed		2.83	0.29
Farm 9	Fodder Radish	plough		2.67	0.14
Farm 9	Fodder Radish	shallow non-inv		2.98	0.22
Farm 10		no till		4.25	0.09
Farm 10	mix-pea	min till		2.93	0.24
Farm 10	mix-Pedders	min till		4.82	0.33
Farm 10	mix-Rigol	min till		3.44	0.57
Farm 10	mix-soil	min till		3.15	0.26
Farm 10	mix-Terra	min till		4.27	0.22
Farm 10	Oil Radish	min till		3.21	0.17
Farm 11	Black Oat & Radish	min till		11.31	0.57
Farm 11		Karat (deep)		9.72	0.64
Farm 11		Karat (shallow)		8.24	0.62
Farm 11		plough		8.17	0.35
Farm 11		straw rake		12.03	0.71

Farm 11	Oil Radish	min till	10.79	0.44
Farm 11	Phacelia	min till	10.58	0.44
Farm 11	White Mustard	min till	9.29	0.16
Farm 12		plough	8.18	0.38
Farm 12	mix	direct drill	8.77	3.54
Farm 12	OSR	direct drill	10.73	0.66
Farm 13	mix	min till	5.64	0.72
Farm 13	mix	plough	4.92	0.04

TABLE 3.9: **Cycle setup and primer sequences for Illumina Sequencing PCRs of the marker genes studied.**

Amplicon	Step	Time and Cycles	Primer	Reference
16S rRNA				
	first round initial denaturation	2 mins 95 °C		Walters et al., 2016
	Denaturation	15 secs 95 °C	515f GTGYCAGCMGCCGCGGTAA	
	Annealing	30 secs 55 °C	806r GGACTACNVGGGTWTCTAAT	
	Elongation	30 secs 72 °C for 25 cycles		
	Final Extension	10 mins 72°C		
	2nd step PCR	8 cycles 55 °C		
fungal ITS				
	first round initial denaturation	3 mins 95 °C		Ihrmark et al., 2012
	Denaturation	15 secs 95 °C	GTGARTCATCGAATCTTTG	
	Annealing	30 secs 52 °C	TCCTCCGCTTATTGATATGC	
	Elongation	30 secs 72 °C for 30 cycles		
	Final Extension	10 mins 72 °C		
	2nd step PCR	8 cycles 55 °C		
18S rRNA				
	first round initial denaturation	2 mins 95 °C		Amaral-Zettler et al., 2009
	Denaturation	15 secs 95 °C		
	Annealing	30 secs 57 °C		
	Elongation	30 secs 72 °C for 30 cycles		
	Final Extension	10 mins 72 °C		

Chapter 4

Critical role of soil pH in restoring ecosystem services in a degraded agricultural soil by chemical and microbial manipulation

4.1 Abstract

Soils, which are degraded by intensive agriculture are thought to be improved by chemical and microbial manipulation in order to restore their functionality. While land use is a known driver of soil microbiome composition, soil pH is the best explanatory variable across scales. In a mesocosm experiment, both, pH manipulation and microbial inoculation from contrasting land use types were applied to a long term bare fallow soil and and their potential to restore ecosystem functions related to C cycling (OM contents, basal respiration, litter degradation (Tea Bag Index), enzymatic activity) and plant growth assessed. Bacterial communities were determined with 16S sequencing from bulk and rhizosphere soil samples, as well as from litter bags and indicator taxa for each land use type determined. Soil pH was strongly influencing the efficacy of microbial inoculation, with best transfer of targeted organisms in high pH soil. Furthermore, pH significantly changed SOM, basal respiration and enzymatic activities. Land use of the transferred microbial communities did not

show any significant effect on the tested functions. Dominant indicators for grassland soil (wash) were chtoniobacterial *Xiphinematobacter* and *DA101* versus archaeal *Nitrososphaerales* and Actinobacteria in cropland soil. These results suggest that manipulation of soil pH can elevate the abundance of target microorganisms, yet their functional significance in delivering ecosystem services remains to be further determined to restore soil health.

4.2 Introduction

Soils provide important ecosystem functions related to human well-being (Dominati, Patterson, and Mackay, 2010), which influence and are influenced by above and below ground biodiversity. To reduce soil degradation, the International Panel on Biodiversity and Ecosystem Services recommends a combination of conservation of species rich natural habitats, sustainable cropland management and restoration of degraded sites to build up resilient soil ecosystem services (Biodiversity and Ecosystem Services, 2018; Biodiversity and Ecosystem Services, 2019). Land management should support synergies of soil functions to maximise multiple ecosystem services. Building up SOM, for example, improves below ground C stocks, plant nutrient status, water holding capacity and biota in soils (Smith et al., 2015).

Soil microorganisms regulate ecosystem services related to C cycling via transformation of plant inputs, biomass turnover and excretion of reactive and adhesive compounds. Consequently, soil microbial activity is often invoked as influencing SOM stocks, and the formation of stable aggregates and improved soil texture (Backer et al., 2018). In addition, soil microbes can influence plants through assisting in plant nutrient acquisition, secreting hormones, secondary metabolites, antibiotics and consequently modulating the response of plants to environmental stress (Backer et al., 2018).

New molecular approaches are now revealing the identities of microbial taxa which respond to land management, but the challenge in translating this knowledge for

applied purposes is in ascribing functions relevant for soil health to these often uncultured indicative taxa (Zhalnina et al., 2013). Though the role of microbial communities in SOM formation and stabilisation got into focus for their potential to improve soil multifunctionality (Kallenbach, Grandy, and Frey, 2016; Cotrufo et al., 2015; Cotrufo et al., 2013; Seaton et al., 2019), it is challenging to prove the role of microbial diversity explicitly from observation and survey. Considering the covariance of biotic and abiotic factors, it is difficult to separate diversity from abiotic effects on soil functionality from soil surveys only (Dequiedt et al., 2009; Griffiths et al., 2011; Griffiths et al., 2016c; Fierer, Bradford, and Jackson, 2007).

Soil pH is the best explanatory variable for bacterial community composition in a range of soil diversity studies, where neutral pH soils show highest bacterial diversity, whereas acidic soils likely harbour fewer, but more dominant taxa (Fierer, Bradford, and Jackson, 2007; Lauber et al., 2008; Lauber et al., 2009; Rousk et al., 2010). Soil pH and other chemical properties like organic matter content co-correlate with ecosystem type and land use, which structure soil microbiomes more than eg. climatic or seasonal conditions (Thomson et al., 2010; Thomson et al., 2015; Malik et al., 2018).

It remains unclear if the increase in organic matter with land use change is caused by change in the microbial community composition or by pH change, added moisture, organic material and plant C inputs (Malik et al., 2018; Jones et al., 2019b). Even in local studies demonstrating associations between high SOM stocks under species rich vegetation with elevated microbial activity and increased root exudate input, the causative role of microbes can not be separated from plant effects (Lange et al., 2015). Moreover, plants steer microbial communities in their rhizosphere through plant specific metabolites and root exudates, which might cover or influence other environmental drivers like soil pH or change soil physical attributes by their morphological traits (Ma et al., 2018; Philippot et al., 2013).

Soil diversity manipulation experiments are therefore explicitly required to directly test the role of microbial diversity in soil functionality (Griffiths et al., 2001; Griffiths et al., 2004; Koziol and James D. Bever, 2016; Bell et al., 2005). Such experiments have found general patterns between species richness and carbon cycling, such as

a positive relationship with litter decomposition and respiration rates (Nielsen et al., 2011), but many relations between biodiversity and functionality remain unclear (Hättenschwiler, Tiunov, and Scheu, 2005; Wertz et al., 2006; Don et al., 2017; Rillig et al., 2015).

It is thus necessary to run controlled experiments that separate biotic from abiotic processes. The experimental transfer of microbial communities from soils of contrasting land use allows this separation and furthermore investigates land management impacts. The challenge in soil transfer experiments remains to reshape microbial communities without changing soil properties too strongly, nor to have a change in microbiome composition covered by the influence of the background/donor soil properties and "hidden treatments" (Huston, 1997). Targeted community manipulation experiments therefore have the potential to yield new information on the functional relevance of microbial land use indicators for soil health, in addition to yielding wider general information on the functional roles of novel soil microbes. There are also potential applied applications, for instance in developing new practices to promote abundances of functionally relevant microbes, or specifically engineering the microbiome for desired benefits (Bell et al., 2005; Wohl, Arora, and Gladstone, 2004).

Indeed, synthetic microbial communities (Großkopf and Soyer, 2014) have been touted as a solution to numerous problems in environmental remediation, waste water treatment, medicine and chemical synthesis (Brenner, You, and Arnold, 2008; Mee and Wang, 2012). They potentially can circumvent the complexity of natural microbiomes and can help to explain specific metabolic functions or species interactions of ecological systems. Difficulties in culturing certain microbial strains make synthetic communities more attractive for applications in smart agriculture, where they were shown to suppress diseases (Hu et al., 2016; Pineda, Kaplan, and Bezemer, 2017) and promote plant growth (Backer et al., 2018; Toju et al., 2018).

For agricultural purposes, it is debatable how *in-vitro* microbiomes can be stably established in the natural world. Microbial communities or consortia are thought to generate stability and robustness toward environmental changes compared to single (culture) species (Brenner, You, and Arnold, 2008; Pineda, Kaplan, and Bezemer, 2017). So far, most diversity manipulation has been generic, eg. inoculation with one

or multiple pure cultures or dilution to extinction approaches (Griffiths et al., 2001; Griffiths et al., 2004) or plant species manipulations (Lange et al., 2015). To date, no studies have tested the performance of synthetic bacterial communities from different land management on ecosystem functionality in a long term bare fallow soil.

Sustainable land use should target enrichment of specific microbial taxa, which are indicative of good soil health and habitats with high SOM content. Undisturbed grassland soils show potentially high biodiversity above and below ground and store high contents of organic matter, and thus likely harbour microbes which fulfil these requirements and help in delivering C cycle related soil functions (Chapter 2). In contrast, croplands are often depleted in their SOM contents as well as in their species richness.

From global soil diversity studies we are beginning to elucidate the identities of dominant taxa to be responsive to land use change (Crowther, Averill, and Maynard, 2019; Hermans et al., 2020; Hermans et al., 2017). However, the role of these organisms in influencing soil health remains unknown, and community manipulation experiments offer an opportunity to specifically test the role of land management influenced communities in directly governing a variety of soil functions.

I previously found consistent microbial indicators of land use change (grassland, restoring grassland and arable), and now seek to use community inoculation experiments to test whether these communities can influence soil functionality. Specifically, I seek to test whether different microbial inocula from arable or grassland soils can benefit a heavily degraded long term bare fallow soil from the Rothamsted long term experiments. Since it is known that other soil properties such as soil pH heavily influence soil microbial communities, I also wish to test the relative importance of community source versus soil pH in influencing both biodiversity and functional responses.

The aim of this experiment is therefore to quantify the possible enhancement of soil ecosystem services of a degraded agricultural soil by chemical and microbial manipulation in form of soil pH adjustment and microbial inoculation.

I hypothesise, that inoculation success will depend on soil condition with respect

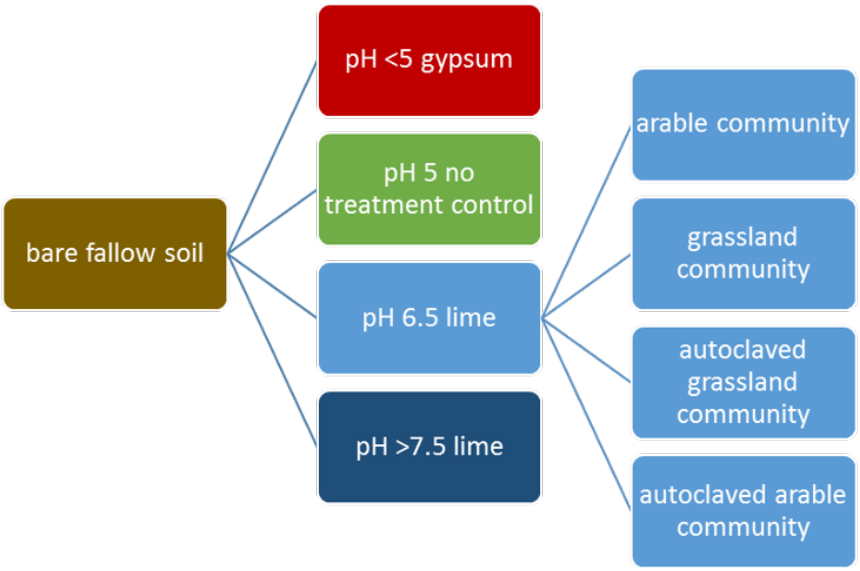


FIGURE 4.1: Experimental design of the soil transfer experiment. A long term bare fallow soil was changed in pH with gypsum $CaSO_4$ and lime $Ca(OH)_2$ to create a pH range from low to high pH. Each pH treatment was then inoculated with microbial communities from arable or grassland soils. To control for hidden effects of inoculation (eg. soil nutrient transfer), autoclaved inocula were simultaneously applied to the pH adjusted soils.

to soil pH. More specifically, I hypothesise that establishment of taxa that are typical/indicative for grassland or arable soils (Chapter 2) will be achieved via inoculation with communities from the respective habitats, and establishment will be accompanied by change in a variety of soil functions. The measurements focus on microbial metabolic activity (respiration and extracellular enzymes), plant biomass production, carbon sequestration, litter decomposition and bacterial diversity.

As I found phylotypes of Verrucomicrobia and Bradyrhizobia to be indicators of grassland soils with high SOM contents, I expect them to be enriched in mesocosms, which were treated with the grassland inoculum and will hence cause the most increase in soil functionality. I equally expect arable indicator taxa, including archaeal Nitrososphaerales to be enriched in mesocosms that were inoculated with arable soil wash.

4.3 Materials and Methods

4.3.1 Sites and sampling

As a background soil for the mesocosms, a field that was maintained as bare-fallow for 50 years at Rothamsted Research, Harpenden Highfield Site, UK was sampled, air-dried and sieved (4mm ^{2}) (Hirsch et al., 2016). This soil is heavily degraded and depleted in carbon stocks by intensive physical disturbance to remove vegetation cover thrice yearly.

As inoculum source for the microbial treatments 4 kg of top soil were sampled at the Parsonage Down SSSI, (at a spot with high plant diversity, personal communication Natural England) for treatment "grassland soil" and "arable" cropland soil from a neighbouring wheat field in May 2018. At each site, 5 replicates were sampled with ethanol sterilised tools into sterile plastic bags and stored in a cooling box until further processing. Distance between the treatments was 1 km and distance between replicates up to 10 m (Figure A.2a).

4.3.2 Soil pH manipulation

After testing pH adjustments in small scale, the Rothamsted bare fallow degraded soil (air-dried, sieved 4 mm), was adjusted in pH. 1 kg of dry soil was thoroughly mixed with 2 g $CaOH_2$ using a hand concrete mixer to perceive treatment "mid", 4 g lime per kg soil for treatment "high" and the equivalent molarity of calcium in form of gypsum for treatment "low" in batches of 22 kg, each. A "control" treatment had no calcium addition or pH adjustment, but the inoculation treatment later on. Soil moisture in the mesocosms was regulated through wicks (non bio-degradable nylon cord), which soaked up distilled water from a reservoir underneath to keep it at a constant level throughout the experiment (Toth, Nurthen, and Chan, 1988).

4.3.3 Inoculation

To transfer the microbial communities, a slurry of 400 g fresh soil per replicate was prepared in 800 ml distilled water (filter sterilised), shaken at 290 rpm for 30 min, let

stand to settle over 4 hours before siphon the supernatant with sterile plastic tubes. 100 ml inoculum were applied per mesocosm containing 1 kg dry, pH adjusted degraded background soil.

To test for the effect of soil wash addition (which may contain low levels of nutrients) without transfer of living microorganisms, half of each soil sample was autoclaved thrice (Howard, Bell, and Kao-Kniffin, 2017) and an inoculum of this applied in the same way as the living treatment. Sterility of the autoclaved soil was tested by growth on full medium agar plates. Filter-sterilised (0.2 um) artificial root exudate solution was added weekly as carbon source to prevent starvation of the microbial communities (Table A.2) (Baudoin, Benizri, and Guckert, 2003). At the beginning of the experiment, chopped, autoclaved straw (0.25 % w/w) was added into the pH adjusted soil substrate in each pot. The amount of straw applied represents the amount necessary to cover the surface of one mesocosm, thus imitating crop residues under real world conditions. Contrarily to the continually supply with artificial root exudates (100 ug C g^{-1} day^{-1}), straw was only added once (2.2 ug C g^{-1} day^{-1}). Furthermore, two tea bags were added per pot, used to measure decomposition rates, contain 1.5 g C (equals 16.7 ug C g^{-1} day^{-1} (Keuskamp et al., 2013)).

4.3.4 Microbial community composition and metabolic activity

To estimate bacterial community composition in the mesocosms, bulk soil *DNA sequencing* of the 16S rRNA amplicon was performed four weeks after inoculation and at the end of the experiment after 12 weeks.

Wheat plants were grown in the soils (following the incubation period for another eight weeks in sub samples) and DNA extracted from the rhizosphere, which I will now refer to as the "root microbiome" (Hu et al., 2018), in contrast to "bulk soil microbiome". Samples were not pooled, but each pot treated as a single replicate (Marotz, 2019).

Soil basal respiration was measured in weeks 3, 5, 11, 13 after inoculation with a portable Infrared-Gas Analyzer type EGM-4 (Steduto et al., 2002). The gas chamber was directly attached to the top of the mesocosm and sealed, where necessary, with

blue tag.

Litter decomposition was tested with the Tea Bag Index (TBI) method. One bag Roiboosh and one Green Sencha were buried 8 cm deep in each mesocosm for the duration of the experiment (Didion et al., 2016; Reed and Martens, 1996). According to Keuskamp et al., 2013, TBI consists of two parameters describing decomposition rate (k) and litter stabilisation factor (S), where k represents short-term dynamics of fresh plant material and S is indicative for long-term carbon storage.

Stabilisation factor $S = 1 - a_g / H_g$

where a_g is the decomposable fraction and H_g is the hydrolysable fraction of green tea and the deviation of the actual decomposed fraction (i.e. limit value) a from the hydrolysable (i.e. chemically labile) fraction H. This deviation can be interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction of plant litter and will be referred to as stabilisation factor S.

4.3.5 Soil potential extracellular enzyme activity

Potential activity of hydrolytic exoenzymes acetase (acetyl esterase, ACE), α-glucosidase (α -GLU), β-glucosidase (β-GLU), chitinase (N -acetyl-b-glucosaminidase, CHIN), phosphatase (PHO) and peptidase (leucine-aminopeptidase, LEU) was assessed with methylumbelliferyl (MUB) and 7-amino-4-methylcoumarin (AMC) conjugated substrates (Sigma-AldrichCompany Ltd, Gillingham, UK), (see Table A.3 for a presentation of all enzymes, their substrates, functions according to Nyyssönen et al., 2013; Weintraub et al., 2007). Enzyme assays were performed on 1.5 g of frozen homogenized soil mixed with 20 ml deionized water in sterile falcon tubes. Samples were shaken for 20 mins at 400 rpm to obtain a homogeneous soil solution; 30 ul soil solution was added to a 96-well microplate containing 170 ul substrate solution at 300 mM (saturated concentration). Reaction plates were incubated in the dark for 3 hr at 28°C with one fluorometric scan every 30 min (BioSpa 8 Automated Incubator, BioTek, Swindon, UK). Fluorescence intensity was measured using a Cytation

5 spectrophotometer (BioTek Swindon, UK) linked to the automated incubator and set to 330 and 342 nm for excitation and 450 and 440 nm for emission for the 4-MUB and the 7-AMC substrate, respectively. For each sample, three technical replicates (soil solution + substrate + water) and a quenching curve (soil solution + water + 4-MUB or 7-AMC) were measured. For each substrate, a control including the 4-MUB- or 7-AMC-linked substrate and water alone were used to check the evolution of fluorescence without enzyme degradation over the duration of the assay. All enzyme activities were calculated in [nkat], the amount (nmol) of catalysed product per second and normalized by g of dry soil (Marx, Wood, and Jarvis, 2001).

To assess bacterial biomass, 250 ul of the soil slurry was mixed with 750 ul water, centrifuged at 1000 g for 5 min, and 500 ul of the supernatant fixed with 500 ul 0.5 % paraformaldehyde solution for storage at -20 °C. All samples were run using the Accuri Flow Cytometer (Becton Dickinson UK Ltd, Wokingham, UK) in deep-well plates after SYBR Green staining and 5 min incubation in the dark as described in (Bressan et al., 2015).

4.3.6 Molecular analysis of the bacterial communities

For DNA extractions, 0.2 g of soil, rhizosphere soil or litter bag material was transferred into 96-well plates and extracted using the PowerSoil® DNA Isolation Kit (QiagenLtd, Manchester, UK). Illumina 2-step amplicon sequencing was conducted according to the protocols of the Earth Microbiome Project (Thompson et al., 2017). In brief, amplicons were prepared using established primers for the 16S rRNA regions (V4-5 region) 515f GTGYCAGCMGCCGCGGTAA and 806r GGACTACNVGGGTWTCTAAT, and PCR protocols (Walters et al., 2016) using high-fidelity DNA polymerase (Q5 Taq, New England Biolabs (UK) Ltd, Hitchin, UK). For purification, PCR products were treated according to manufacturer's instructions with Zymo DNA Clean up Kit (Zymo Research Europe GmbH, Breisgau, Germany). In a second round of PCR, Illumina adapters were added and all samples normalized using the SequalPrep ™ Normalization Kit (Thermo Fisher Scientific Ltd, Altrincham,UK), pooled and concentration verified spectrophotometrically with Qubit (Thermo Fisher Scientific Ltd, Altrincham, UK). Illumina high-throughput sequencing was performed

with MiSeq® Reagent Kit V3, which is capable of producing 2 × 300 bp paired-end reads (Illumina Ltd, Cambridge, UK).

Illumina sequencing output was analysed with DADA2 (Callahan et al., 2016) in R (R Core Team, 2017), to demultiplex raw sequences and trim paired sequences to uniform lengths. The core sequence-variant inference algorithm was applied with the DADA function to dereplicated data before paired-end sequences were merged and chimeras were removed. Taxonomic data were assigned from GreenGenes for bacterial taxonomy (DeSantis et al., 2006). The 16S phylotype abundance table was rarefied to 10000 reads to account for differences in sampling depth, before assessing β-diversity in non-metric multidimensional scaling ordinations and running Permutational Multivariate Analysis of Variance (PERMANOVA) with the functions in vegan (Oksanen, 2008). Significant ($p < 0.05$) indicator phylotypes for Pristine grassland and Arable soil (wash) treatments were determined using the indval routine in labdsv (Dufrene et al., 2011) and wider statistical analysis and visualization was performed in R version 3.6.0 using the packages ggplot2 (Hadley Wickham, 2016) and labdsv (Roberts, 2019).

4.3.7 Plant growth and plant associated bacterial communities

After destructive sampling at the end of the experiment, 2 * 250 g aliquots fresh soil were transferred from each mesocosm into two potting bags with drainage holes, which were individually placed into sterile trays to avoid cross contamination of microbial communities. Three wheat seeds (KWS Alderon, 97 % germination rate) per pot were applied 1 cm deep in the wet soil and the pots watered three times weekly for ten weeks. Maximum plant height was measured as distance from soil to the longest leaf after eight weeks growth. Above ground biomass of the wheat seedlings was estimated fresh and dry after 24 hours drying at 60 ^{o}C. Root material was treated for rhizosphere community analysis according to (Liu et al., 2018). In brief, root material from all seedlings was separated manually from loosely adhered soil and about 2 g fresh plant material pooled in 50 ml tubes containing 25 ml sterile 0.1 M PBS buffer, shaken at 250 rpm for 5 min and rhizosphere soil centrifuged for

9 min at 4000 g after removal of the roots. Supernatant was discarded and the soil pellet stored at -20 ^oC.

4.3.8 Soil chemical and physical attributes

Soil pH, Soil moisture and Organic Matter contents

After the experiment, all mesocosms were sampled destructively and sieved (2mm) prior to further analysis or storage at -20 ^oC for enzymatic and microbial diversity analysis. Soil pH was determined in a solution of 10 g fresh soil in 25 ml distilled water. Organic matter content was measured as Loss-on-Ignition. In brief, samples were oven dried at 105 ^oC until the weight was stabilised to estimate soil moisture and further incinerated at 430 ^oC for 16 hours (Reed and Martens, 1996).

4.3.9 Statistical Analysis

Indicator OTUs derived from Illumina amplicon sequencing were calculated with the simper and indval functions in the R! packages labdsv, vegan and ade4 for bacterial and fungal OTUs, respectively (Dufrene et al., 2011). The indicator value of species i for class j is obtained with the equation:

$$IndVal_{ij} = 100 \cdot A_{ij} \cdot B_{ij}$$

A_{ij} is specificity, i.e. the proportion of the individuals of species i that are in class j

B_{ij} is fidelity, i.e. the proportion of sites in class j that contain species i. This index is maximum (=100 %) when the individuals of species i are observed in all sites of only one site group. Only significant indicators (p-value 0.01) were taken into consideration for further analysis.

Simper values describe the similarity percentage based on the Bray-Curtis-dissimilarity.

Potential EEA was calculated as slope of fluorescence intensity (light emission) over time until maximum enzyme activity (saturation point V_{max}) according to Marx, Wood, and Jarvis, 2001.

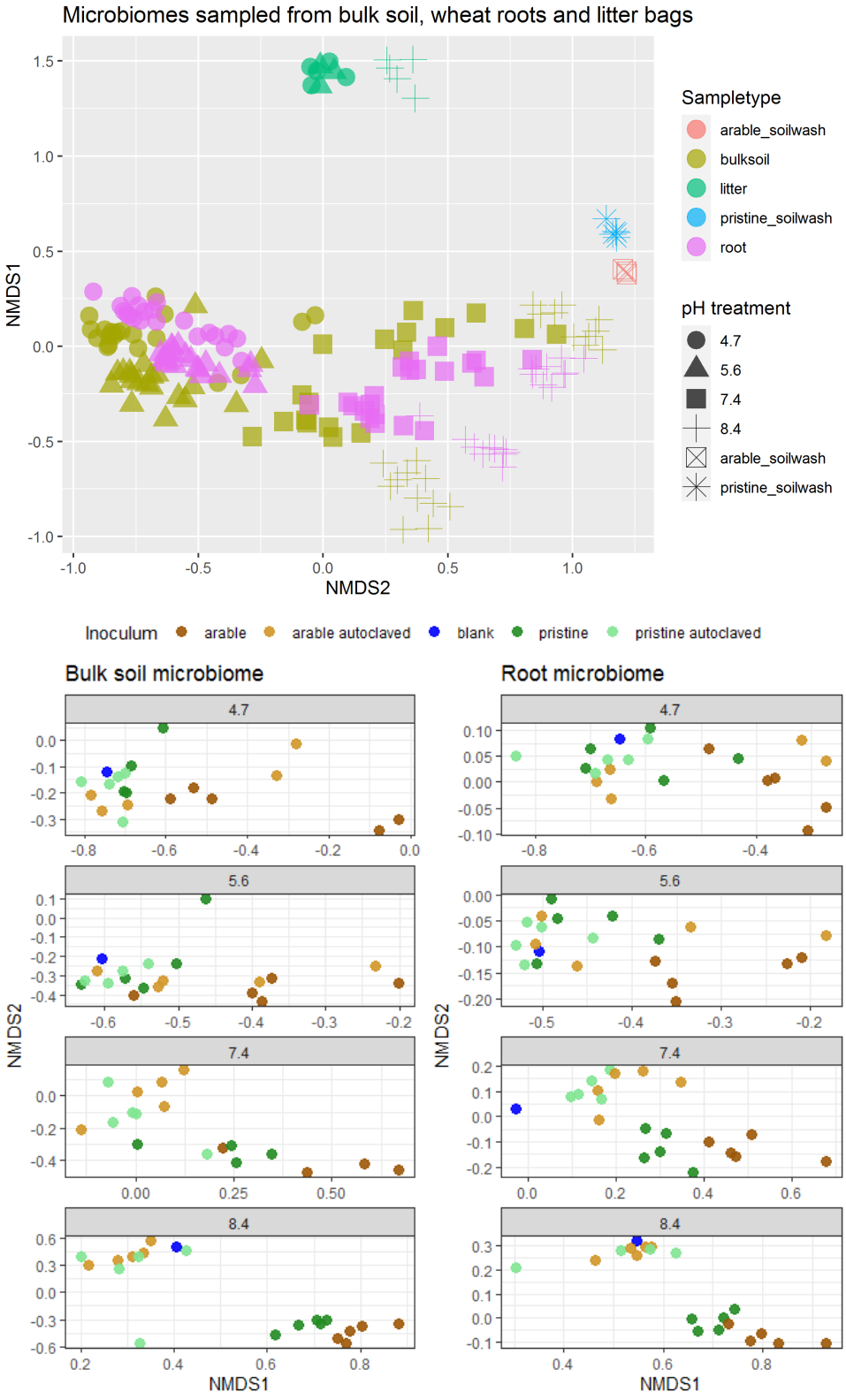


FIGURE 4.2: **Bacterial community composition in all samples** Top: NMDS plot of the microbial communities in mesocosm bulk soil (yellow green), rhizosphere of wheat plants in the same mesocosm soil (violet), soil wash for transfer of inoculum onto the degraded (long term bare fallow) soil (blue = pristine, red = arable) and from green tea litter bags (green), which were buried for eight weeks in each mesocosm to test for decompositon. Clustering according to pH and source of sample is apparent. Bottom: NMDS plots of bulk (left panel) and rhizosphere (right panel) split by pH treatment group. Dark colours indicate mesocosms that received living inoculum, while lighter shades represent the autoclaved inoculum.

4.4 Results

4.4.1 Establishment of inoculated soil microbial communities

Sequencing of the bacterial DNA in the bulk soil after 12 weeks, as well as from 15 litter bags (12 weeks after burial) and the wheat rhizosphere (after another eight weeks of plant growth) revealed strong interactive effects of soil pH and inocula source on microbial community establishment. NMDS ordinations showed distinct clustering of sampled communities according to land use type, soil pH and source inocula (either alive or autoclaved) (Figure 4.2, Table 4.1).

TABLE 4.1: **PERMANOVA testing effects of soil bacterial community composition as a function of the experimental treatments**: inoculation with soil wash from contrasting land use (grassland/arable), autoclaving the soil wash before inoculation (yes/no), pH adjustment of the degraded background soil and origin of the sample (bulk soil/ wheat root / soil wash used for inoculation/ litter bags).

	Df	SumsOfSqs	MeanSqs	F.Model	R^2	p	
sampletype	2	7.67	3.84	18.26	0.12	0.001	***
pH	5	18.04	3.61	17.17	**0.28**	0.001	***
autoclaving	1	1.93	1.93	9.20	0.03	0.001	***
land use	2	1.23	0.62	2.93	0.02	0.001	***
Residuals	172	36.14	0.21		0.56		
Total	182	65.02			1.00		

Bacterial community composition in bulk and root samples was very similar across pH treatments, with pH being the strongest discriminator and sample type (DNA extracted from "soil wash", "bulk soil", "root soil" or "litter") the second strongest, followed by autoclaving of the inoculum (“treatmentcontrol”) and land use. Hereafter, I refer to "land use" as the source of inoculum it was derived from. This was confirmed following multivariate permutation testing (Table 4.1).

Additionally, analyses of diversity metrics (not shown) confirms that α-diversity (species numbers per sample rarefied to 5000 reads) is clearly higher in alkaline soils, whereas we find fewer taxa, which are more dominant, in acidic soils (ANOVA for species richness: $F_{\text{Sampletype, 2 df}} = 148.4$, $F_{\text{autoclaving, 1 df}} = 34.4$, $F_{\text{pH, 5 df}} = 28.0$, $F_{\text{land use, 2 df}} = 22.97$, $p < 0.0001$ for each).

Microbiomes from the “blank” control pots, which had no addition of soil wash,

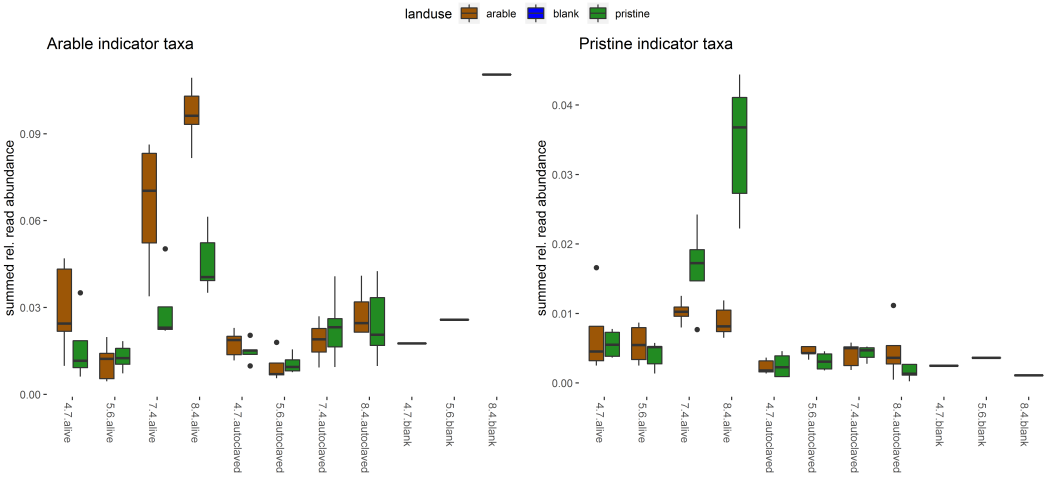
cluster in the NMDS with autoclaved treatments (Figure 4.2). The wash from autoclaved soils served as control for the addition of nutrients with inoculation without transferring a living microbiome.

The strongest separation of bacterial communities according to land use is found under the high pH conditions with living inoculum (Table 4.4). Furthermore, land use differences are stronger in living inoculations than for pots that received a sterile inoculum, supporting the efficacy of the inoculation approach.

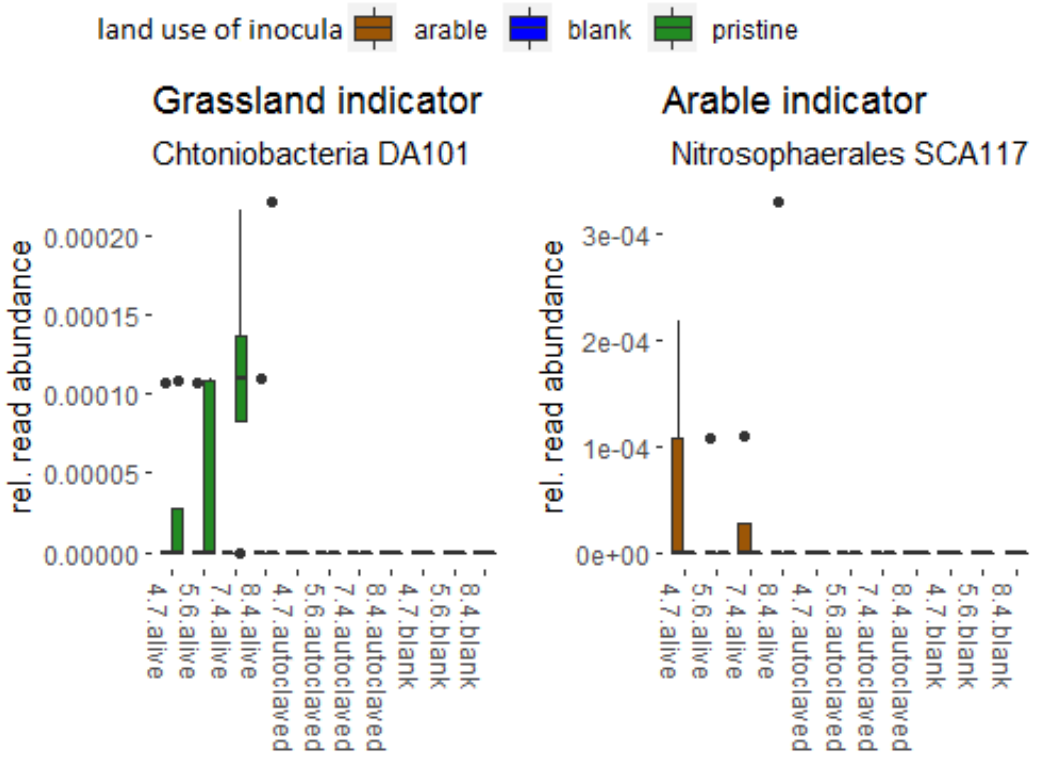
The NMDS separation between living and autoclaved soil communities suggests a successful treatment effect. Still, this is no proof of effective transfer of targeted microorganisms which are indicative of pristine or arable soils.

4.4.2 Bacterial indicators of arable and pristine land use

In order to create contrasting microbial communities in the mesocosms with inoculation, I first had to validate contrasting community compositions in the inoculation sources (pristine/arable soil wash). I performed an indicator analysis on the sequenced communities in the soil wash which was used to transfer microbial communities from donor soil/inoculum to mesocosm. In total, 1669 indicator ASVs were found (in the wash), of which 235 taxa indicated arable and 158 pristine land use (see SI table A.5 for top most abundant taxa). The two top most abundant pristine grassland indicators are verrucomicrobial DA101, which were previously defined as pristine land use indicators, whereas actinobacterial *Arthrobacter psychrolactophilus* and archaeal *Candidatus Nitrososphaera SCA1170* were top indicators for arable (Chapter 2). Other arable soil indicators were ASVs from Methylibium, Nitrospira, genus Thermomonas, family Chitinophagaceae, family Sinobacteraceae, whereas grassland soil is indicated by specific members of Chthoniobacter, genus Arenimonas, Rhodoplanes (Rhizobiales) and genus Kaistobacter. To check if the inoculated microbes established and recovered in the degraded bare fallow background soil, summed relative abundances of all aggregated significant indicator taxa (ASVs) for each land use were then calculated for each mesocosm treatment (Figures 4.3a and 4.3b).



(A)



(B)

FIGURE 4.3: **Effects of inocula on different types of soil.** Relative abundance (summed) of bacterial taxa in mesocosms inoculated with microbial communities from grassland (green) and arable (brown) land, and where no inoculum was applied ("blank", blue). Indicators were determined from soil wash to transfer communities into degraded agricultural soil. Left: bacterial taxa determined as indicative of land use "arable", right: bacterial taxa determined as indicative of land use "pristine".

Clearly, autoclaved inocula did not change the relative abundances of arable or pristine indicators in the same way as the living treatments (Table 4.2). In samples receiving inoculum with living communities, arable indicator organisms were enriched in arable treatments, where we observed the strongest effect in low pH, mid pH and high pH soils. Equivalent results were observed for pristine indicators, which furthermore showed the highest variation in high pH samples (Table 4.4).

4.4.3 Microbial inoculation and soil pH effects on soil functions

The following soil functions were tested for their dependence on land use and soil pH. In order to assess specific effects of the above determined indicator bacteria, statistical analysis (ANOVA, t-test) was performed on living treatments only, which were enriched in their relative abundances of these taxa.

Primary production

In soils with neutral pH, treatments with arable community inocula showed significantly lower wheat germination than pristine ones (Figure 4.5 and A.1). Germination rate of wheat seedlings was independent from land use in most treatments. Most seeds (96%) germinated in autoclaved arable treatments in pH 5.6, and pH 7.4 with autoclaved pristine soil wash (average 95%). More wheat seedlings germinated in low pH soils, while the lowest number of wheat seedlings per pot was 53% in soil pH 7.4 with arable inoculation. Disregarding soil pH and autoclaving, more seeds germinated in pristine than in arable treatments, (arable mean: 76%, pristine mean 86%, t-test t = -2.1, df = 63.4, p - value = 0.039).

Due to the difference in total number of wheat plants, I calculated above ground biomass both, per pot and per plant. Plant biomass was lowest in soils without gypsum or lime addition, regardless of land use. Most dry mass grew in high pH soils with autoclaved, pristine inoculum (1.1 g per pot) and second most with 0.93 g per pot in pristine/alive/acidic soil. Per plant, we found the largest dry mass in alkaline soils with autoclaved arable community (0.21 +/- 0.05 g), whereas the lightweight of all plants was found in 7.4, pristine, alive (0.112 g). Plant height, evaluated after

8 weeks of growth, was similarly highest in low pH and high pH treatments and differences became visible (photo A.1).

Soil basal respiration and SOM content

Respiration rates increased with increasing pH (Figure 4.5). Adjustment of soil pH significantly influenced respiration (ANOVA F = 64.5, p-value < 0.001). For mesocosms that got treated with living soil communities, respiration rates were higher in arable than in pristine treatments across all pH groups, although these differences were not statistically significant (Mixed Effects Model Chi^2=0.0023, p = 0.96).

Soil organic matter content was significantly influenced by pH (for all samples: ANOVA-F = 21.05, p < 0.001, for "alive" inoculum only: F = 4.01, p = 0.052)), but not by autoclaving the soil wash (3.59 % in alive, autoclaved 3.58 %, t-test t = 0.01, df = 52.8, p-value = 0.99), nor by land use (t = -0.29, df = 50.3, p-value = 0.8).

Litter decomposition

The Tea Bag Index indicates soil C stabilisation and litter decomposition. Both, decomposition rate k and Stabilisation factor S, were not significantly influenced by inoculation nor pH adjustment (Mixed Effects Model with pH as fixed effect: ANOVA - p = 0.13 and 0.49, respectively). Nevertheless, there is a trend of pristine soil communities showing higher C Stabilisation factors than arable ones (Figure 4.5, Table 4.6). Stabilisation factors were higher in acidic and high pH soils than in neutral and control pH. In these two extreme pH treatments, pristine communities tended to have higher C Stabilisation factors than the arable treatments (high pH: arable= 0.23, pristine = 0.25, in low pH: arable = 0.25, pristine = 0.27), which was not the case in sterile control inoculations. Overall, land use had no significant effect on the Tea Bag Index. Indicator analysis of the litter bag bacterial communities was carried out separately (Indicator table Annex A.5). There was an enrichment of fast growing, copiotrophs of the classes α- and β- Proteobacteria on green tea litter and no indicators of the phyla Verruchomicrobia, Nitrospirae and Acidobacteria.

Surprisingly, the bacteria specific 16S rRNA amplicon sequencing detected a high

abundance of fungal mitochondrial DNA in litter, which is associated with Aspergillus (NCBI BLAST).

Enzymatic activities

Soil enzyme activities all were strongly influenced by pH (Figure 4.4). Acetase activity was higher in pots that got inoculated with pristine soil communities, but no significant differences were observed (ANOVA $p > 0.3$). Apart from acetase, all other tested enzyme activities were clearly increasing with decreasing pH (Figure 4.4). In order to assess the influence of indicator bacteria on soil functions, only samples that were treated with "alive" inoculation were considered, none of the evaluated enzymes were significantly dependent on land use, whereas there were clear relations with soil pH (Table 4.5). The strongest interactions with soil pH were found in β - glucosidase, peptidase, phosphatase, chitinase (p - val <0.001) and α - glucosidase (p - val 0.04) activities.

TABLE 4.2: **Analysis of Variance table: Recovery (relative read abundance) of soil wash indicator taxa from contrasting land use as function of soil pH and autoclaving of the soil wash.**

indicators of arable soil wash						**pristine soil wash**					
living inoculum						living inoculum					
	Df	Sum Sq	Mean Sq	F value	p		Df	Sum Sq	Mean Sq	F value	p
low pH	1	0.004	0.004	3.357	0.110	**low**	1	0.002	0.002	24.068	0.002 **
Residuals	7	0.008	0.001			Residuals	7	0.001	0.000		
control pH	1	0.008	0.008	15.746	0.004 **	**control**	1	0.004	0.004	35.332	0.000 ***
Residuals	8	0.004	0.001			Residuals	8	0.001	0.000		
mid pH	1	0.024	0.024	24.499	0.003 **	**mid**	1	0.002	0.002	37.625	0.001 **
Residuals	6	0.006	0.001			Residuals	6	0.000	0.000		
high pH	1	0.085	0.085	119.031	0.000 ***	**high**	1	0.006	0.006	65.205	0.000 ***
Residuals	8	0.006	0.001			Residuals	8	0.001	0.000		
autoclaved inoculum						autoclaved inoculum					
arable						pristine					
pH						pH					
low pH	1	0.000	0.000	2.541	0.150	**low**	1	0.001	0.001	9.716	0.014 *
Residuals	8	0.001	0.000			Residuals	8	0.001	0.000		
control pH	1	0.005	0.005	2.677	0.146	**control**	1	0.002	0.002	9.495	0.018 *
Residuals	7	0.012	0.002			Residuals	7	0.001	0.000		
mid pH	1	0.000	0.000	0.083	0.780	**mid**	1	0.000	0.000	7.408	0.026 .
Residuals	8	0.003	0.000			Residuals	8	0.000	0.000		
high pH	1	0.009	0.009	9.232	0.016 *	**high**	1	0.000	0.000	7.051	0.029 .
Residuals	8	0.008	0.001			Residuals	8	0.000	0.000		

TABLE 4.3: **Analysis of Variance table: summed abundance of arable (upper) and pristine (lower) bacterial indicators as a function of soil pH, land use of transferred microbiome and autoclaving.**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
arable indicators						
land use	2	0.00	0.00	6.17	0.003	***
pH	1	0.02	0.02	53.57	<.001	***
autoclaving	1	0.01	0.01	25.14	<.001	***
Residuals	74	0.03	0.00			
pristine indicators						
land use	2	0.00	0.00	3.02	0.0551	
pH	1	0.00	0.00	16.06	<.001	***
autoclaving	1	0.00	0.00	28.42	<.001	***
Residuals	74	0.00	0.00			

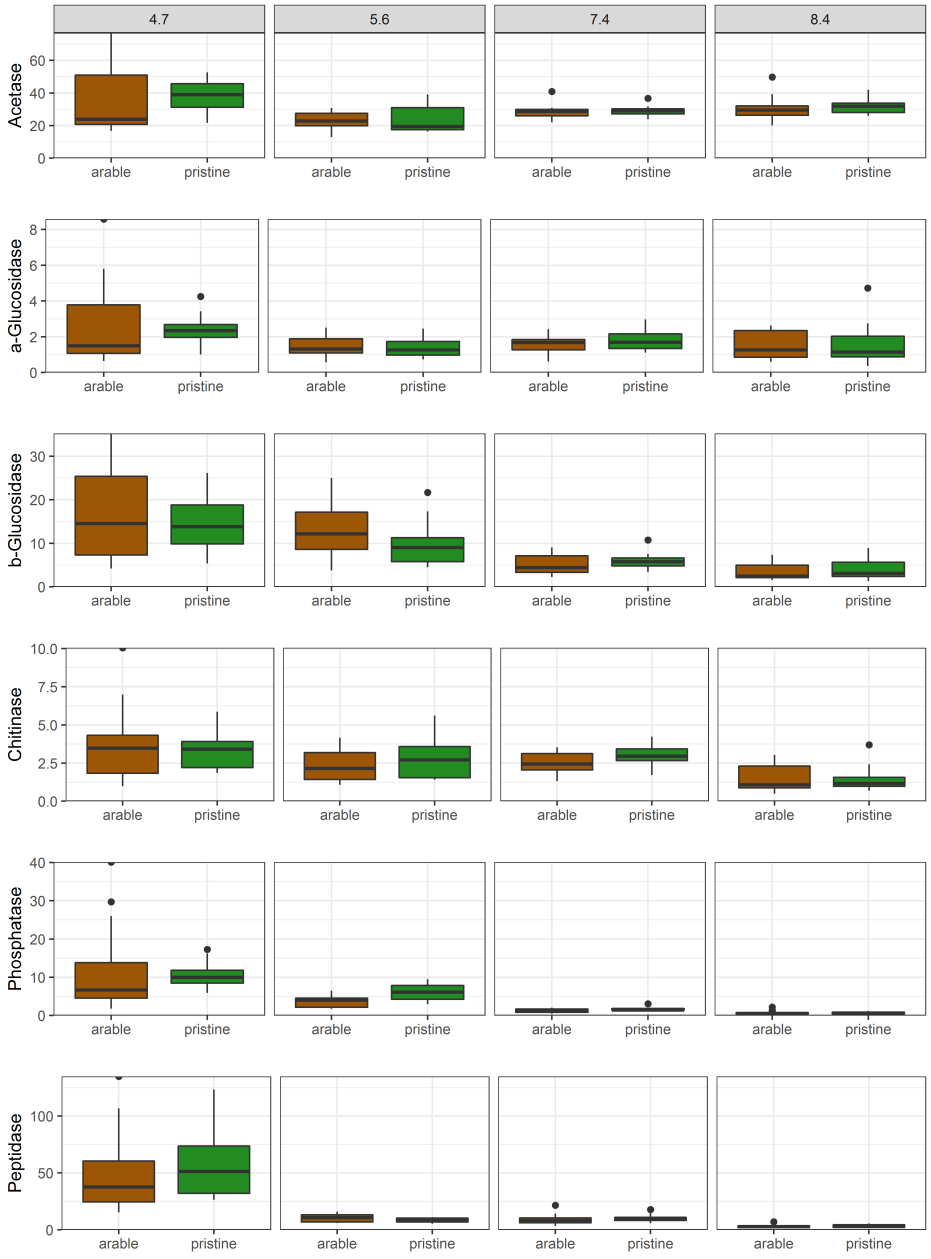


FIGURE 4.4: Box-Whisker-plots displaying soil extracellular enzymatic activity of acetase, α-glucosidase, β-glucosidase, chitinase, phosphatase and peptidase in [nmol *s^{-1}] per g soil. Samples were separated per soil pH treatment from acidic pH 4.7 (left panels) to alkaline pH 8.4 (right panels). Brown boxes indicate arable, green boxes pristine land use of the inoculated microbial communities. None of the tested enzymes were significantly changed by land use (ANOVA p-value >0.05)

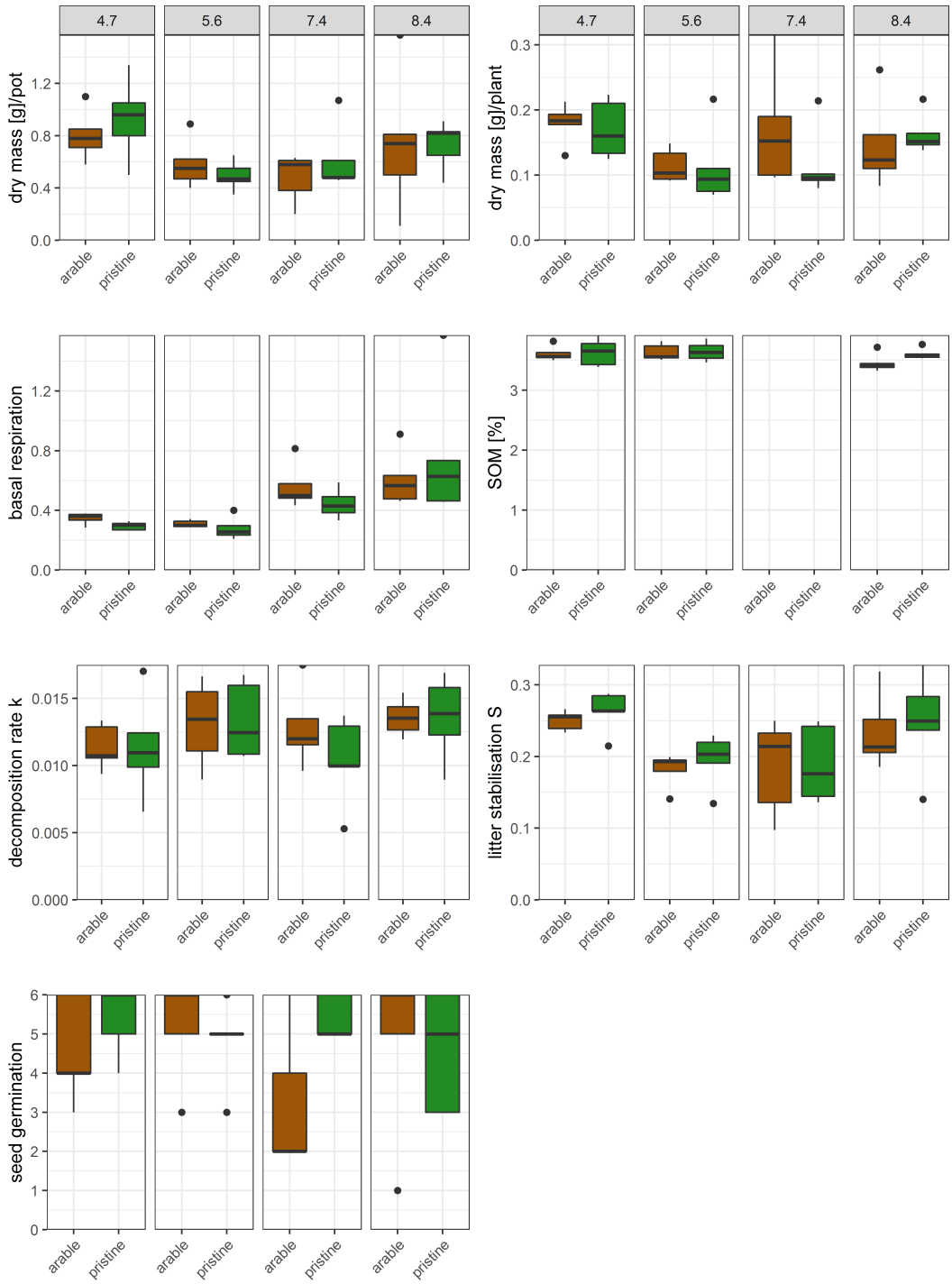


FIGURE 4.5: Effects of soil pH (4.7 to 8.4) and land use (brown: arable; green: pristine) of microbial community transferred into arable background soil on *plant growth* (above ground biomass per individual plant (upper left) and per pot with six seeds (upper right), *soil basal respiration* cumulative rates from four time points [umol CO_2 / m^2*sec], *decomposition* rate k and *litter stabilisation* factor S (TBI consists of two parameters describing decomposition rate (k) and litter stabilisation factor (S), where k represents short-term dynamics of fresh plant material and S is indicative for long-term carbon storage. Stabilisation factor $S = 1 - a_g / H_g$ where a_g is the decomposable fraction and H_g is the hydrolysable fraction of green tea and the deviation of the actual decomposed fraction (i.e. limit value) a from the hydrolysable (i.e. chemically labile) fraction H. This deviation can be interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction of plant litter and will be referred to as stabilisation factor S.), *soil organic matter* content [%], *Triticum germination* (number of seeds germinated out of six seeds). Displayed are samples, that were inoculated with living soil wash, whereas autoclaved soil wash alias "controls" are excluded, as consequence of molecular diversity results.

TABLE 4.4: ANOSIM results of bacterial community similarity as a response to land use (pristine vs. arable inoculation) and pH

pH	ANOSIM R	p	ANOSIM R	p	
	not autoclaved		autoclaved		
bulk and root					
low (4.7)	0.119719	0.1	0.053311	0.251	
control (5.6)	-0.03273	0.596	0.029111	0.242	
mid (7.4)	0.849826	0.001 ***	0.789784	0.001	***
high (8.4)	**0.949815**	0.001 ***	**0.891628**	0.001	***
bulk soil					
low	0.1	0.2	0.08	0.263	
control	0.516	0.022 *	**0.1875**	0.134	
mid	0.677083	0.033 *	0.09375	0.232	
high	**0.936**	0.011 *	-0.02	0.578	
root					
low	0.76875	0.007 **	-0.0125	0.457	
control	0.516	0.015 *	0.004	0.466	
mid	0.9125	0.008 **	0.004	0.487	
high	**0.98125**	0.011 *	**0.363636**	0.143	

4.5 Discussion

Sustainable agricultural management requires detailed understanding of soil microbial processes to combat land use change driven soil degradation. The aim of this mesocosm experiment was to define the effects of microbial communities from contrasting land use on soil functions related to carbon cycling across a pH gradient. The microbiomes of bulk and rhizosphere soil were modified via pH adjustments and transfer of targeted members of microbial communities from a species-rich grassland or an intensive cropland. The experiment furthermore confirms that artificial root exudates did not reshape the bacterial communities more than surrounding soil properties (Dennis, Miller, and Hirsch, 2010). Although the same exudate solution was supplied to each mesocosm, soil microbial communities still differed between treatments. The hypothesised differential change in bacterial community composition of soils from contrasting land use was confirmed by the dominance of indicator

TABLE 4.5: Analysis of Variance of soil potential extracellular enzyme activities as a function of land use (pristine grassland/arable) and soil pH categories (low/control/mid/high). Only ALIVE samples are considered in the analysis.

	ALIVE samples	Df	Sum Sq	Mean Sq	F value	p-value	
β - Glucosidase	soil pH	1	1502.91	1502.91	36.52	< 0.001	***
	land use	1	3.42	3.42	0.08	0.77	
	pH * land use	1	5.69	5.69	0.14	0.71	
	Residuals	53	2181.3	41.16			
Peptidase	soil pH	1	12935.8	12935.8	30.11	< 0.001	***
	land use	1	137.46	137.46	0.32	0.57	
	pH * land use	1	12.52	12.52	0.03	0.87	
	Residuals	51	21908.17	429.57			
Phosphatase	soil pH	1	862.77	862.77	25.9	< 0.001	***
	land use	1	0.7	0.7	0.02	0.89	
	pH * land use	1	6.05	6.05	0.18	0.67	
	Residuals	52	1732.22	33.31			
Chitinase	soil pH	1	43.76	43.76	21.06	< 0.001	***
	land use	1	2.58	2.58	1.24	0.27	
	pH * land use	1	0.51	0.51	0.25	0.62	
	Residuals	53	110.14	2.08			
α- Glucosidase	soil pH	1	7.56	7.56	4.5	0.04	*
	land use	1	0.08	0.08	0.04	0.83	
	pH * land use	1	2.12	2.12	1.26	0.27	
	Residuals	53	89.05	1.68			
Acetase	soil pH	1	142.59	142.59	1	0.32	
	land use	1	40.59	40.59	0.29	0.6	
	pH * land use	1	70.39	70.39	0.49	0.48	
	Residuals	53	7539.07	142.25			

TABLE 4.6: ANOVA tables for soil organic matter content, soil basal respiration, Tea Bag Index, plant growth and seedling germination as functions of land use of inoculation and soil pH (function ∼ land use * pH). Only ALIVE samples are included in this table.

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		Df	Sum Sq	Mean Sq	F value	p-value	
SOM	pH	1	0.083225	0.083225	4.181	0.048	*
	land use	1	0.051926	0.051926	2.609	0.115	
	pH * land use	1	0.051086	0.051086	2.567	0.117	
	Residuals	38	0.756358	0.019904			
soil basal respiration	pH	1	0.987809	0.987809	33.021	< 0.001	***
	land use	1	3.88E-05	3.88E-05	0.001	0.971	
	pH * land use	1	0.032924	0.032924	1.101	0.299	
	Residuals	49	1.465823	0.029915			
S_stabilisation	pH	1	0.001035	0.001035	0.359	0.552	
	land use	1	0.003431	0.003431	1.189	0.281	
	pH * land use	1	0.001671	0.001671	0.579	0.450	
	Residuals	52	0.150086	0.002886			
k_decomposition	pH	1	1.17E-05	1.17E-05	1.470	0.231	
	land use	1	6.63E-06	6.63E-06	0.835	0.365	
	pH * land use	1	3.12E-06	3.12E-06	0.392	0.534	
	Residuals	52	0.000413	7.95E-06			
plant biomass (total)	pH	1	0.017625	0.017625	0.206	0.652	
	land use	1	0.000447	0.000447	0.005	0.943	
	pH * land use	1	0.026428	0.026428	0.309	0.581	
	Residuals	54	4.617459	0.085508			
biomass (per plant)	pH	1	2.52E-06	2.52E-06	0.001	0.977	
	land use	1	0.001231	0.001231	0.406	0.527	
	pH * land use	1	7.86E-05	7.86E-05	0.026	0.873	
	Residuals	54	0.163558	0.003029			
seed germination	pH	1	1.011208	1.011208	0.511	0.478	
	land use	1	0.956734	0.956734	0.483	0.489	
	pH * land use	1	0.638017	0.638017	0.322	0.573	
	Residuals	54	106.9113	1.979839			
plant height	pH	1	7.828312	7.828312	0.385	0.538	
	land use	1	27.4298	27.4298	1.349	0.251	
	pH * land use	1	8.203876	8.203876	0.403	0.528	
	Residuals	54	1098.356	20.33993			

taxa belonging to the chtoniobacterial *Xiphinematobacter* and DA101 in pristine grassland versus archaeal *Nitrososphaerales* and Actinobacteria in arable soils. Those communities were then successfully transferred onto mesocosms containing a degraded organic matter depleted long term bare fallow soil.

Experimental manipulation of indicator bacteria

Strikingly, I observed a recovery of exactly those soil prokaryotes, which were defined as indicative for the respective land use in the pots twelve weeks after the start of the experiment and they are in line with the microorganisms previously found in pristine/arable soils (Chapter 2). Although being very distinct for each land use type, these indicator taxa were not dominating their equivalent microbiomes. The efficacy of microbiome transfer was strongly dependent on soil pH. Soil pH, manipulated via liming was previously shown to be a significant determinator of bacterial community growth and activity in reciprocal experiments (Pettersson and Bååth, 2013). In alkaline soil conditions, microbial communities clustered the strongest according to land use (Figure 4.2, Table 4.4).

Land use specific inoculation effects increased with increasing pH, where differences between arable and grassland soil inocula became more apparent. This could be due to the high soil pH of the donor habitats from which the transferred bacteria were derived, resulting in a "home-field advantage" of organisms already adapted to high pH conditions (Don et al., 2017). This could also explain the elevated relative abundance of indicator taxa in soils which were manipulated in pH, but not inoculated ("BLANK") (Figure 4.3a). Another possible explanation is the generally higher level of biodiversity/species richness in high pH soils (Rousk et al., 2010), providing a habitat for a wider range of species, thus creating the opportunity for target species to re-establish once they got transferred. Moreover, the toxicity of eg. Aluminium and heavy metals is dependent on the bio-availability and solubility of these compounds in a medium, which is usually given at low pH (Jones et al., 2019b; Lu et al., 2020).

Effect of indicator microbes on soil functionality

Though I found a strong influence of soil pH on all tested functions, there was little to no effect of inocula derived from different land use on the investigated soil functions. This confirms previous experimental studies, which showed, similarly, no consistent effects of species richness on soil respiration, decomposition or microbial activity, nor correlations between altered microbial community structure and functional stability (Griffiths et al., 2001; Griffiths et al., 2004). Collated soil diversity manipulation experiments found a tendency of species richness and C cycling in highly diverse (more than 10 species) systems, including positive relationships with biomass, litter decomposition and respiration (Nielsen et al., 2011), while Wertz et al., 2006 found no reduced respiration with species loss.

Enzyme activities are driven by pH, but not inoculum

In a variety of environments, soil pH and organic matter steers enzymatic activities, with β-GLU, LEU, PHO, CHIN and α-GLU increasing in their activities with increasing SOM stocks (Sinsabaugh et al., 2008; Šťursová and Baldrian, 2011; Stark, Männistö, and Eskelinen, 2014). Due to the removal of dead plant material and tillage caused increased soil respiration, organic material in croplands is drastically reduced compared to grasslands and forests, as are the corresponding degrading enzymes (Tscherko and Kandeler, 1999; Saviozzi et al., 2001). It is furthermore known that hydrolytic enzyme activities in temperate soils correlate positively with soil pH, but acid phosphatase negatively (Acosta-Martinez and Tabatabai, 2000). Hydrolytic enzymes degrade labile compounds, while recalcitrant material is rather subject to oxidative enzymes. Hydrolytic enzymes are a function of substrate availability, pH and stoichiometry of microbial nutrient demand (Sinsabaugh et al., 2008). In the presented experiment, potential activities of β-GLU, LEU, PHO, CHIN and α-GLU were significantly influenced by pH, but not land use, which might be explained by the pH specificity of excreted enzymes, the pH specificity of the enzyme excreting microbial communities or by the enzyme assay itself. Stark, Männistö, and Eskelinen, 2014 identified strong effects of liming and fertilization on β-GLU in acidic,

nutrient poor Tundra soils, whereas LEU and α - GLU were not significantly influenced. There, β-GLU and phenol oxidase activities increased with decreasing soil pH, similarly to the pH effects I observed in my experiment with calcareous temperate grassland and agricultural soil. The results above corroborate the view that there is no direct, linear relationship between soil ecosystem functions and ecosystem structure. Community coalescence, the mixing of entire microbial communities, does not lead to additive effects on functionality, but rather to synergistic and antagonistic effects depending on the types of communities brought together and the new environmental conditions emerging from mixing the substrates (Rillig et al., 2015; Don et al., 2017). A recent pot experiment tested the effects of alkaline vs. acidic /organic amendments to ameliorate heavy metal polluted, acidified agricultural soil and found increased SOM, plant performance of *Lactuca sativa* (lettuce) and nutrient availability in the high pH treatments (Lu et al., 2020).

Plant growth increases with Calcium addition

In my experiment, most plant biomass grew in soils with pH adjustment, which was likely caused by the higher content of the plant nutrient Calcium in the gypsum and lime amendments. The growth optimum for *Triticum* is defined between pH 6.4 and 7.0, where micronutrients are easily available to the plant. While I added $Ca(OH)_2$ to increase soil pH, lime in form of chalk $CaCO_3$ or limestone is commonly applied on agricultural soils, which often results in increased microbial activity and crop yields, higher SOM and increased aggregation (Bronick and Lal, 2005). As I aimed to specifically investigate soil C cycling, incorporation of carbonate was avoided to prevent hidden treatments (Huston, 1997). Sulphates were added to the low pH pots in form of gypsum $CaSO_4$, likely to change microbial nutrient cycling related to sulphur, which was not evaluated.

Bacterial diversity is pH driven and requires specific genetic adaptation

The highest bacterial α-diversity was detected in high pH mesocosms, which is a typical pattern for soil communities (Wan et al., 2020). In contrast, Malik et al., 2017 found higher taxonomic α- and γ-diversity in low pH soils, but functional diversity based on metagenome analysis was not reduced compared to alkaline habitats. There, functional genes related to respiration were less frequent in alkaline low organic matter soils compared to C rich acidic ones. Moreover, genes related to carbohydrate-, amino acid-, phosphorus- and sulphur metabolisms were more common in high pH environments and genetic responses to stress are likely an adaptation to drought and physical disturbance in intensively managed cropland (Compared to my experiment, Malik and colleagues examined soils of contrasting pH and contrasting edaphic and management properties, with high pH soils being rather C depleted croplands and low pH soils resembling undisturbed environments with high SOM stocks (Malik et al., 2017)).

4.5.1 Limitations

Other soil inoculation experiments used sterile media or artificial mineral soil to transfer microbial communities into. I decided not to sterilise the bare fallow soil, as the abundance and activity of microorganisms was reportedly very low (Hirsch et al., 2009). Furthermore, soil nutrients are changed by both, γ -radiation and heat based sterilisation methods, causing hidden treatments in the bare fallow soil. One could argue that in real agricultural settings, the degraded soil will not be sterile and transferred/inoculated microorganisms will have to out compete the indigenous community, too, making the experimental design actually more realistic and applicable to real world conditions.

Regarding the Tea Bag Index as litter decomposition method, Hättenschwiler, Tiunov, and Scheu, 2005 investigated the diversity of litter species on microbial decomposition and criticised experiments testing the unrealistic event of a single plant species litter. Although one might argue that green tea and roiboosh are unlikely to be found

in temperate agricultural settings, I appreciate the opportunity of comparing experimental results with globally derived data from the Tea Bag Index (Keuskamp et al., 2013). In accordance to this issue, another limitation is the very simplified root exudate solution, which was the sole fresh C an N source throughout the experiment, not resembling the highly diverse input of compounds in a species rich grassland, although we know that root exudates are crucial in structuring soil bacterial communities (Dennis, Miller, and Hirsch, 2010).

Microbiome structures in the wheat rhizosphere were strongly changed by soil treatments, surprisingly more so than by the plants. Clearly, more research needs to be conducted with different crops and different soil types to start with. The results of this experiment support the view, that soil microbial communities can be manipulated to once, increase the abundance of targeted organisms and second to maintain and enhance the soil functions related to carbon cycling. More soil functions should be considered testing, like particle structure and organic matter stability, using stable carbon isotope labelled substrates. While plant growth in this assay was only tested on a broad level, plant health could be assessed as chlorophyll and nutrient contents, photosynthetic activity or grain yield. Furthermore, effects of soil pH and land use could be studied on mesobiota, considering eukaryotic and fungal soil organisms.

4.6 Conclusion

The manipulation of bacterial soil communities in this pot experiment underlined the non linear relationships between their taxonomic diversity and ecosystem functions related to C cycling. While pH had significant effects on enzymatic potential activities, SOM and basal respiration, inoculation with microbial communities from different land use was not significantly changing soil functions. Moreover, other soil functions including plant growth, litter decomposition (Tea Bag Index) and acetase activity were not significantly changed by the treatments. Likewise, plant performance in neutral soils was lower than in calcium amended ones, but not significantly, without being clearly influenced by microbial community composition or

land use. However, both pH and inoculation significantly affected soil community composition.

Soil pH, driver of microbiome composition in the environment, was determining the efficacy of microbiome transfer via inoculation, with indicator taxa re-establishing best in high pH soils. Strikingly, amongst those indicators were the same taxa as found in large scale survey (Armbruster et al., 2020; Zhalnina et al., 2014). Dominant indicator taxa in the soil wash were the chtoniobacterial *Xiphinematobacter* and *DA101* in pristine grassland versus archaeal *Nitrososphaerales* and Actinobacteria in arable soils. This leads to the conclusion, that soil microbial diversity can be manipulated through pH to elevate the relative abundance of target microorganisms, which in turn support or deliver the desired soil services.

Nevertheless, it remains to be confirmed which bacterial taxa belong to the group steering soil functionality, considering ecosystem services beyond the agricultural context. This experiment tested only very broad ecosystem functions and future studies should thus consider specific soil functions of interest in relation to the indicator organisms, eg. focus on the nitrogen cycle with ammonia-oxidising Thaumarcheota and nitrogen fixing Verrucomicrobia. In order to maintain and restore soil health, the findings of this experiment suggest a thorough consideration and/or control of soil pH instead of microbiome transfer for the optimal microbiome composition.

Chapter 5

Discussion and recommendations for future research

5.1 Introduction

Land use driven loss of soil organic matter and associated reduction of biodiversity and ecosystem functionality are a challenge for present and future human generations. It is thus necessary, to protect healthy soils from human over-exploitation and restore areas, which are already degraded. To say it with the words of business experts: "We can't manage what we can't measure." (Peter Drucker). We therefore need reliable indicators to track the state of soils and biological diversity across highly diverse and heterogeneous environments and the many forms of disturbance, which terrestrial ecosystems face. A number of recent studies elucidated key microbial taxa being specifically associated to land use and/ or soil conditions (Zhalnina et al., 2013; Liddicoat et al., 2019; Cerecetto et al., 2021).

However, there is still no consensus on whether there are functionally consistent responses of soil microbial taxa to land use driven change in microbial community composition. In this chapter, the experimental work presented in Chapters 2 - 4 is summarised and findings are synthesised in relation to the initial objectives of the thesis project. The objectives were to

- define molecular microbial indicators of land use (change) in soil nucleic acids,

- test the recovery of such indicator microorganisms in a restoration chronosequence,
- evaluate the effectiveness in soil carbon sequestration and biodiversity of Conservation Agriculture in British farm soils,
- explore the interactions of soil chemical properties, especially pH, with microbial diversity and ecosystem services,
- experimentally manipulate soils to enrich/deplete targeted members of microbial communities and establish their relative roles and soil functionality.

Here, I present the findings in regard to the overall aims and wider implications for future research, which are summarised in schematic Figure 5.1.

5.2 Synthesis of results

5.2.1 Impact of land management on soil and soil microbial communities

Land use is a known driver of change in soil nutrients (Smith et al., 2016) and soil microbial community composition (Zhalnina et al., 2013; Thompson et al., 2017; Liddicoat et al., 2019). However, soils are highly variable systems and their responses to environmental change depend on both, intrinsic and extrinsic factors (like soil properties related to bedrock material and pedogenesis in the first, plant cover and fertiliser application in the latter case). While microbial communities are known to be impacted by land use, wider consequences for microbial functions remain unknown.

In the catchment scale survey presented in Chapter 2, I found paired land-use contrasts along a grassland restoration chronosequence revealed the strong impact of management intensity on soil nutrients, enzymatic potential activities and bacterial and fungal biodiversity. Here, organic matter, as well as Mg, total C and N contents were consistently larger in grasslands than in arable fields, while plant available phosphorus concentrations were increased in fertilised cropland. In this

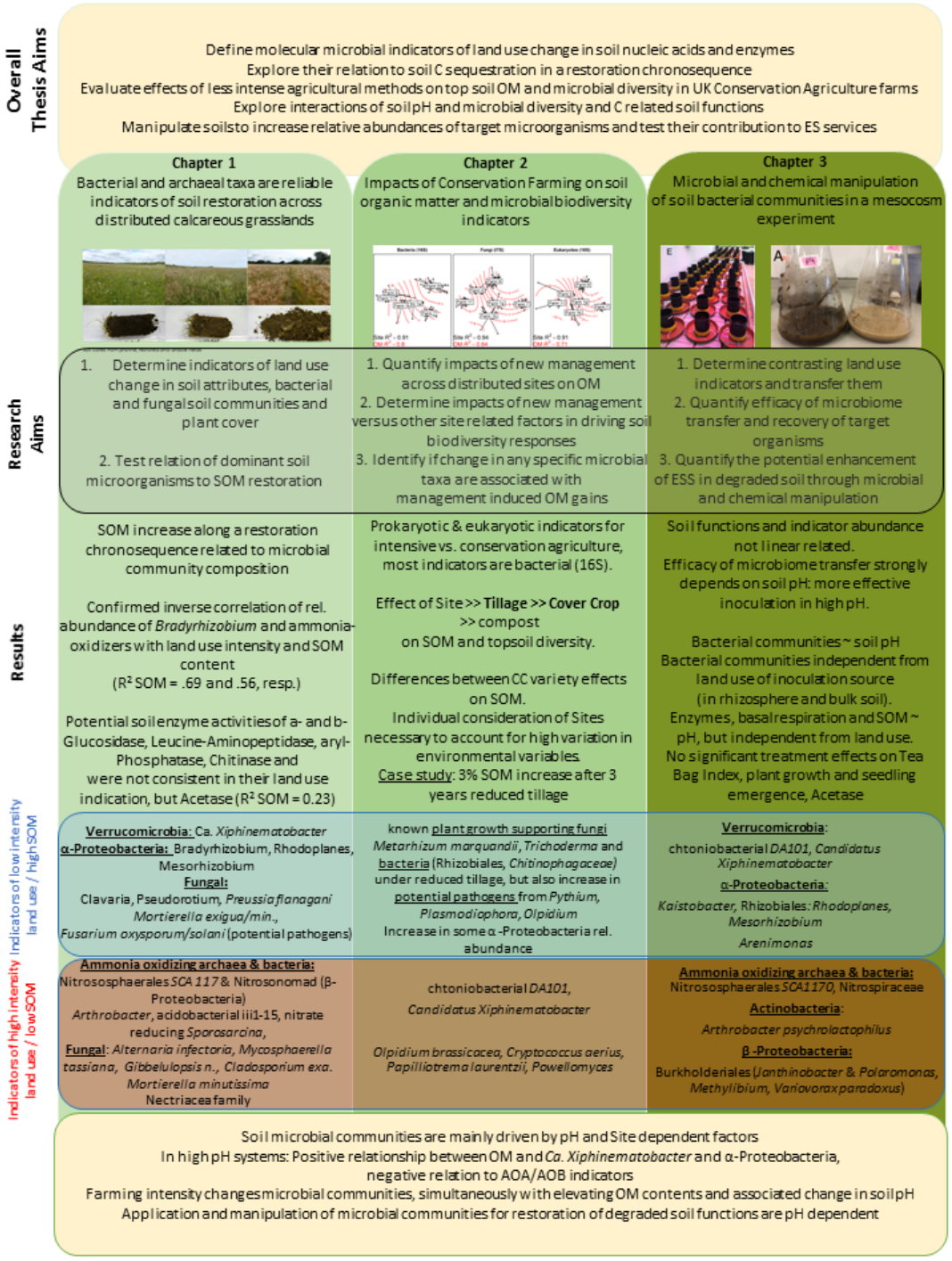


FIGURE 5.1: Schematic of thesis structure, aims and main findings and conclusions.

unique landscape scale survey which was restricted to high pH systems on calcareous bedrock material, I was specifically able to address the impacts of management while minimising the often confounding effect of pH change - which is known to be strongly impacted by management, and a main driver of microbial biodiversity (Bahram et al., 2018; Griffiths et al., 2011; Fierer and Jackson, 2006). The use of this system revealed management related soil organic matter content was actually determining community structures with R^2=0.85 for bacteria and R^2=0.53 for fungal NMDS scores. In the chronosequence, SOM contents increased with time of grassland restoration and with the restoration of former arable field, all microbial communities and soil properties converged towards levels found in pristine grassland soils.

Though soil extracellular enzymes influence soil nutrient cycling and are thus related to SOM and land use category, enzymatic activities showed no consistent indication for either land use, nor for specific microbial community structures in the chronosequence (Chapter 2). Only Acetase was significantly associated to land use, but the extremely high variance of enzyme activities here did not lead to significant results. Overall, these results suggest that potential extracellular enzyme activities are less reliable and suitable soil quality indicators than nucleic acids from bacterial and fungal communities. These findings were furthermore upheld by the results of Chapter 4, were land use had no significant effect on the tested suite of extracellular enzyme activities in soil mesocosms, which were inoculated with bacterial communities derived from arable vs. grassland soil. Instead of land use it was soil pH, that changed the potential activities of β-Glucosidase, Leucineamino-Peptidase, Phosphatase, Chitinase, α-Glucosidase in the experiment significantly. Surprisingly, this was not the case for Acetase activities, which were less affected in the experiment in contrast to the findings of Chapter 2.

With respect to new agronomic practices, I found that conservation management also increased SOM on many less intense managed plots in the Conservation Agriculture survey Chapter 3. All six farms which tested tillage as a treatment factor in their experiments, observed higher levels of OM in their top soils with min till or no till in comparison to conventional ploughing. This was regardless of crop rotation,

(cover) crop species, duration of alternative management or addition of organic supplements. However, there was no consistent soil amelioration observed from cover cropping, as soils under different CC varieties created both, higher and lower OM stocks compared to the control treatments. Importantly, it has to be considered that only the top 15 cm are commonly sampled, when soils are ploughed to 30 cm depth. This could clearly obscure C accumulation rates, as greater C contents are to be expected near the surface (Powlson et al., 2012). As microbial communities in the top horizon of the soil column differ from those in deeper layers (Griffiths et al., 2000; Baldrian et al., 2012), a depth profile of the microbial community composition down to 30 cm and below would be highly interesting to follow the C accumulation after physical perturbation or novel tillage practice but is challenging to sample and costly to analyse.

Moreover, it remains a difficulty to extrapolate from single sampling points to a whole field, due to the heterogeneous nature of soils. For agricultural purposes, usually, several soil samples from one field are pooled to reduce costs of chemical analyses (Wollum, 1994) and the results in this thesis suggest that soil cores from the same field cluster in similarity of both, their microbial communities and SOM contents. In the landscape scale farm survey (Chapter 3), the effect of geographic location was much stronger than any management effect on both, topsoil OM and biodiversity. Separate extraction and analysis of replicated soil cores confirmed a clustering of samples according to their location and homogeneity of the replicates. This calls for a careful and precise assessment of soil benefits from conservation agriculture, which accounts for the natural environmental variance alongside any management effect.

5.2.2 Soil microbial taxa as land use indicators

Fierer, Wood, and Mesquita, 2020 recently reviewed the potential of soil microbial communities to assess soil health as cheap and high throughput alternative to otherwise costly soil analyses (eg. soil nutrients, functional properties, pollutants). However, the usefulness of microbial indicators also depends on the cost and effort required to measure the indicated variable, eg. pH sensitive bacteria can detect soil

pH, which is much cheaper to assess directly with an electrode, while complex soil contamination with heavy metals and organic pollutants are costly to assess and hence worth to be replaced by microbial-based assays, which will furthermore account for bio-availability and toxicity of such substances. The authors point out that microbial communities in soil are temporarily variable, but not too variable to track changes in soil parameters. Thanks to the immense diversity of soil microbes, it is possible to characterise a multitude of environmental parameters with microbial metrics. To use microbial taxa, genes or metabolic activities as indicators, consistency in their indicative meaning needs to be ensured by a thorough selection of soils and ecosystems in a widely distributed area (Hermans et al., 2017; Fierer, Wood, and Mesquita, 2020).

Such a selective, spacial distributed approach was applied in the restoration chronosequence, which was limited to calcareous systems across a catchment area of South Britain. Moreover, the British Farm survey on Conservation Agriculture Chapter 3, which included a wider variety of soil types and differential intensity in agricultural management was following these guidelines and used as a test system to validate the results from Chapter 2. In the former, I determined bacterial taxa belonging to *Bradyrhizobium*, *Rhodoplanes*, *Mesorhizobium* and verrucomicrobial *DA101* and *Ca. Xiphinematobacter* as indicators for species-rich, complex, high SOM ecosystems, which were inverse correlated in their relative abundance to ammonia oxidising bacteria and archaea including *Nitrososphaerales SCA117, Nitrosonomad, Arthrobacter*, acidobacterial *iii1-15* and nitrate reducing *Sporosarcina*.

Additionally to the bacterial indicator taxa, I also determined fungal indicators for both land use types. In Chapter 2, these were primarily comprised by cosmopolitan saprotrophic taxa, with *Clavaria, Pseudorotium, Preussia flanagani, Mortierella exigua/minutissima* being elevated in undisturbed grasslands, in contrast to *Alternaria infectoria, Mycosphaerella tassiana, Gibbelulopsis n., Cladosporium exa., Mortierella minutissima* and a member of the Nectriacea family being specific to arable soils. The indicators for SOM rich grasslands included potential plant pathogenic *Fusarium oxysporum* and *Fusarium solani*, which is unexpected but possible, as *Fusarium* species are known to be associated with grasses (*Poacea*) (Dinolfo, Castañares, and Stenglein, 2017). In line with this paradox, potential crop pathogenic *Pythium*, *Plasmodiophora*

and *Olpidium* taxa were indicative for less intense agricultural management (Chapter 3). In this case, I want to point out that it was only one Site which used as example and the findings should be interpreted cautiously.

However, we also found plant growth promoting bacteria and fungi to be enriched under conservation agriculture, including *Metarhizum marquandii*, *Trichoderma*, Rhizobiales and Chitinophagaceae, as well as some members of the α-Proteobacteria. As the functional capacity, like potential pathogenicity, remains a conundrum for many microbial species, either experimental testing under controlled conditions according to Koch's postulates will be required to confirm diseases that these species cause to crops. From the molecular biological perspective, it would be highly interesting to sequence whole genomes of the potential pathogens for their actual genetic capacity to infect crops. Moreover, a combination with actual crop health assessments in soils, which have been under min till/ CC management would confirm the effects from a more applied farming perspective (Helander et al., 2018).

As a general finding in the first two chapters, it was found that prokarote 16S rRNA amplicon sequencing delivered most indicator taxa for the relevant land use category, more than detected with the eukaryotic 18S or ITS marker genes. This simply could be due to ubiquity, with greater numbers of the same bacterial phylotypes being found across treatments. Specific fungal and protist taxa are potentially more spatially variable and may therefore not be detected as indicators due to issues of rarity. Alternatively, due to the physical disturbance of soil structure and function in agricultural systems, I expect lower trophic levels to dominate the soil biota, with prokaryotic bacteria and archaea being more abundant than fungal or protist organisms (per gram of soil) (Bardgett, Hobbs, and Frostegård, 1996).

Higher fungal-to-bacterial biomass ratios are typical for less intensive managed soils (Bardgett, Hobbs, and Frostegård, 1996), likely caused by physical disturbance of fungal mycelium from tillage on the one hand and the higher C-to-N ratio of fungi compared to bacteria, which are therefore favouring substrates with higher C-to-N ratios. A cropland however, is likely to be enriched in fertiliser derived nitrogen compounds, and so lower C-to-N ratio may favour prokarya.

The ecosystem stoichiometry may therefore also be relevant in explaining the importance of different indicators, which is determined by the nutrient inputs into the system, which are primarily provided by vascular plants into the ground (Spohn, 2016). In strong dependence of the composition of litter and root exudates, that get channelled into the ground, soil microbial communities are structured accordingly and nutrient cycling and soil C turnover times are changed (Trumbore, 2000).

Together, the combined results show that molecular technologies are powerful tools for monitoring soil status, condition and health, either in restoration land use or novel management contexts. I was able to demonstrate that consistent microbial indicators exist across similar soil systems in similar climates and same parent material. However, there remain large challenges, both in terms of synthesising and disseminating these indicators, but also extending their meaning to other soil systems - ie. in acidic or not near neutral communities. It is highly likely that these indicators will not be as responsive to management and SOM in other soil systems. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity as almost half of extracted soil prokaryote and fungal DNA is possible to be originated from dead cells and extracellular DNA. The results of Carini et al., 2016 highlight that, although the effects of relic DNA are variable across different soil types, it is especially important to account for relic DNA in acidic soils, or in soils with few exchangeable base cations (K+ and Ca^{2+} below ca. 40 meq 100 g^{-1}). Comparisons of soil DNA and RNA as proxy for the total vs. active microbial communities give hints that there are similar levels of diversity with different community compositions (Gkarmiri et al., 2017; Baldrian et al., 2012; Griffiths et al., 2000) and more research is necessary to quantify these differences. In general, relic DNA is expected to influence results of studies in environments, where negromass is abundant because of either low abundance of active microorganisms or factors hindering the decomposition (Carini et al., 2016).

Thanks to technological advances, dissemination of these results was possible in an online platform (https://shiny-apps.ceh.ac.uk/ID-TaxER/), in which prokaryote indicator taxa of pristine grassland vs. arable soils from Chapter 2 have been included. To complement this list of microbial indicators, broader surveys with similar contrasts across different systems are needed, but also, predictive models which

will predict likely indicators for different land uses under different geo-climatic contexts.

With respect to indicators of novel management, clearly also the indicators will vary across soils and land uses. Combined with a wide variety of novel management practices, it is clear from my work showing site specific context dependent effects, that to truly evaluate efficacy, then long term consistent experiments are required across different soils under different geo-climatic context.

More generally, molecular tools can be used to monitor ecological status of soil ecosystems. These tools are not limited to microbes. Vascular plant DNA targeted amplicon sequencing offers a replacement of traditional vegetation surveys in the long term, with clear advantages over the common plant cover assessments: Vegetation surveys only allow a snapshot of the situation at the moment of assessment. But once DNA is extracted from soil samples, it can be stored for a long time and be re-assessed if necessary and this could furthermore be used to describe plant communities from the past, as long as there is frozen soil or DNA stored (Fahner et al., 2016).

5.2.3 Managing soil microbes to increase soil ecosystem services

It remains to be determined, how to best manipulate soil properties and microbial communities with their specialists, in order to restore degraded soils and to enhance soil multi-functionality and resistance to disturbance. The molecular methods providing taxonomic indicators give no information about ecosystem functions. In order to relate specific organisms to ecosystem services, it is important to translate the indicators to soil functions.

In Chapter 4, I did an experiment to test functional effects of altered indicator abundance across a soil pH gradient and with microbial communities from contrasting land use intensity. Additionally, this allowed an examination of the efficacy of inoculation under different soil conditions for establishing desired communities - a topic of current applied interest.

Reliable monitoring of soil ecosystems and their recovery after disturbance requires consistent, universal indicators (Urbanski et al., 2018). Many approaches have been

assessed to remediate degraded arable fields into healthy soil of which pH manipulating amendments (Lu et al., 2020; Rowley, Grand, and Verrecchia, 2017) and inoculation with microbial communities (Wubs et al., 2016; Pywell et al., 2007) are two promising tools. In the mesocosm experiment in Chapter 4, I inoculated a degraded arable soil with two microbial communities from contrasting land use in order to assess their contributions to specific C related soil functions. It is noteworthy, though, that my experiment aimed to test if indicator taxa found in the field surveys could be manipulated under laboratory conditions instead of specifically trying to ameliorate the long term bare fallow soil.

To successfully change soil bacterial communities and influence the ecosystem services they support, it is most important to manage soil for the right pH, as it was shown in the experiment in Chapter 4. However, an inoculation with microbial communities was no reliable method to restore functionality of degraded long term bare fallow soil in Chapter 4, but as mentioned, that was not the aim of the experiment. While the transfer success of a microbial community was largely driven by soil pH, with high pH soils offering best potential for re-establishment of the original taxa from the inoculum. This might be explained by the origin of the transferred community, which was from high pH soils, too. These findings confirm results from microbial biogeography studies, where factors such as pH are directly responsible for selective processes operating on bacterial taxa (Fierer and Jackson, 2006; Griffiths et al., 2011). Unfortunately, the inoculation did not appear to affect any of the soil functions measured despite the small but significant community effects, and throughout the thesis there were limited associations between taxonomic changes and measures of enzymes and gross functional process, such as Tea Bag Index and soil respiration. Soil pH amendments were shown to be an effective treatment of acidified, heavy metal polluted soil, to improve enzyme activity, SOM contents, lettuce growth (laboratory conditions) and bacterial responses, with alkaline $CaMgPO_4$ and limestone additions being more effective than acidic, organic additions like biochar and manure compost (Lu et al., 2020). Moreover, an application of "synthetic" or specifically designed microbial communities is thought to solve the problem of reduced yields in conservation agriculture (Bender, Wagg, and Heijden, 2016), but no improvements

in wheat growth or seed germination was observed in my case. The use of more specific and advanced functional approaches in the mesocosm experiment in Chapter 4 may have resolved change occurring in more specific processes.

Linking indicator taxa with soil functions

In laboratory cultures, slow-growing, specialist taxa are quickly out competed by fast growing generalists, leaving knowledge gaps on whole phylogenetic branches in the tree of life. Recently, advances in pure cultures progressed the study of the widespread phylum of Acidobacteria, where a member of Subdivision 6 was isolated for the first time although its globally abundant (Huber et al., 2016). The paradigm that only 1 % of the sequenced bacteria are growing under artificial conditions was recently questioned (Martiny, 2019), as microbial cultivation techniques progressed substantially and about 52% of sequences and 34% of taxa (defined as more than 97% 16S rRNA phylogenetic similarity) have a known cultured relative. With this technological progress, microbial lifestyles and metabolic traits of specific microorganisms can be explored in detail to understand their functional capacity and contribution to ESS and create databases which combine taxonomic and functional information. The falling cost of DNA sequencing and improved computational methods furthermore enabled the application of MAGs (metagenome assembled genomes) to environmental samples (Quince et al., 2017).

Another possible approach linking phylogeny and function are single-cell genomics, which are based on the extraction of cells from the matrix prior nucleic acid extraction and allow distinct characterisation of microbial traits (Kalisky and Quake, 2011). The high throughput sequencing platforms underwent an evolution themselves, starting with 454 pyrosequencing, over IonTorrent, Illumina to PacBio and the Oxford Nanopore technologies and future methodological progress may hence increase our ability to sequence larger samples sets at high resolution and quality (Shendure et al., 2017). Similarly, metatranscriptomics and metaproteomics aim at the resolution of the whole community transcriptome or proteome, respectively, thus providing functional information (Baldrian and López-Mondéjar, 2014).

A main limitation in all these methods will be the computational analysis of such

complex data and unbiased, reproducible and standardised bioinformatics (Quince et al., 2017), but they will undoubtedly shed light on functions of novel taxa.

Molecular approaches towards sustainable land use

Plant gene editing with CRISPR/CAS9 are touted to help with crop production, especially under drought or nutrient depleted conditions. However, just as with genetically modified plants (GMO), long term effects on natural and human systems are yet to be observed. Once the genome of an organism is edited, it will reproduce with the newly designed genes what is somewhat of a violation against the gene pool which all living beings share. Personally, I would prefer less invasive methods to reach sustainability in agriculture and land use. This could be achieved by enhanced biodiversity in anthropogenic systems, while manipulating targeted organismic groups which deliver specific, desired ecological functions (Bender, Wagg, and Heijden, 2016).

The results in Chapter 3 suggest that tillage strongly affects both, SOM contents and microbial communities, stronger than cover cropping or addition of compost/manure. Reduced tillage approaches were able to increase OM stocks in all tested farms implementing tillage as treatment in their experiment in this conservation agriculture survey, which is supporting the findings of the systematic review investigating no-till and min-till effects on soil C from Haddaway et al., 2017. However, the scientific literature is full of evidence for benefits by crop diversification through cover cropping, agro-forestry and field margins on above ground biodiversity and associated ecosystem services, as well as on soil microbial properties (Kim et al., 2020). Sustainable soil management is expected to mitigate climate change especially when focus is set to soil C sequestration in agricultural areas and land with a history of C loss due to land use change (Amelung et al., 2020). The high site dependence of management extensification efficacy in Chapter 3 pushes us to individual assessments of soils ecosystem services, as it is now possible with digital solutions. To date, land use adaptations and lifestyle preferences of distinct microbial taxa can be modelled and mapped thanks to big data computing and intelligent algorithms making use of large scale survey data (Jones et al., 2019a). In an agricultural context, farmers want

to know if their management practice leads to improved biodiversity, eg. more nematode biomass or higher species richness. There is however a significant problem with deriving meaningful metrics of microbial diversity from molecular approaches, as it has been consistently shown that alpha diversity of bacteria is higher in arable soils than in natural systems (George et al., 2019), which could be due to latent DNA from dead microorganisms, which might be proportionally more eminent than DNA from active prokaryotes. This leads to the conclusion, that bacterial species numbers alone are not a good indicator of soil health. Instead, the change of specific indicator taxa and community composition serves as a metric for soil functionality. It is undeniable, that long lists of microbial taxa are not truly helpful to the farming community. Most of those taxa are unknown in their function, while previously identified and cultured microorganisms with reported beneficial or pathogenic properties can confuse, as a known "pathogen" is not necessarily harmful to the crop. Only combinations of soil molecular microbiology with crop health assessments would confirm the effects of novel management on soil and crop health (Helander et al., 2018). However, a scorecard system including indicator taxa as multiple metrics (as presented in Chapter 3) was rated helpful by agronomists but needs to be tailored to the management, climate and soil type of each individual farm. Currently, tools are being developed using model-based predictions, which still need to include molecular biodiversity data (https://robiwangriff.shinyapps.io/soil-map-app).

Nevertheless, I believe that soil ecological engineering of microbial communities is able to mitigate a trade-off between high yields in conventional intensive agriculture and sustainability with reduced profit margins in conservation agriculture and organic farming.

Hence, I recommend a smart selection of soils which are source of an inoculum, which considers especially pH and the specific adaptations of the microbial communities therein and their metabolisms, in the case that a soil inoculation is desired - if not for restorative, but for scientific purposes. Therefore, for future applications which seek to manipulate microbial communities (either through inoculation of beneficial /synthetic communities, or rhizosphere engineering) it is extremely important to consider the soil properties to which they will be inoculated, both in terms of

targeting specific taxa and also in monitoring and manipulating the soil conditions for optimal success.

5.2.4 Future work and my vision of combining new technologies

Evaluation of the relevance of the indicators detected in this thesis requires comparison to other sites and soil systems (Guerra et al., 2020). While my thesis has focused on British calcareous grasslands in Chapter 2 and agricultural soils in Chapter 3, other regions and land-use types have to be assessed. Metagenomic data derived from other soil surveys should be compared to validate the results, especially when there is functional information available. However, only replicated, spatially distributed LTEs allow quantitative comparisons between different sites. Ideally, these cover a pH gradient, as pH was shown to be a main driver of microbial community composition.

In future studies, ESS should be assessed with more sophisticated functional assays, as mentioned in Section 5.2.2: metabolomics, transcriptomics and the measurement of volatile organic compounds from soils deliver insights into the actual metabolic activity of soil organisms. Soil microbial enzymes, assessed in this thesis via buffer assays, can also be evaluated via the sequencing of enzyme marker genes to compare the genetic capacity with the enzymatic activity. While this work focused on carbon-related functions, the methodologies applied here could be expanded to include the sulphur, nitrogen and phosphorus cycles. Quantification of metabolic rates and a mass balance can be realised when isotopic tracers are included to follow soil nutrient cycling and the relative contribution of microbial communities in transforming and stabilising processes (Gkarmiri et al., 2017; Cotrufo et al., 2015).

Climate change will increase the frequency of extreme weather events in the future, and their impacts on soil ecosystem services and microbial communities should be further investigated across different land-uses. Simulating droughts and floods experimentally as one part and integrating national monitoring data will likely deliver valuable insights into the responses of soil biology to disturbance events for soils with different land-use histories. Additionally, other disturbance factors have to

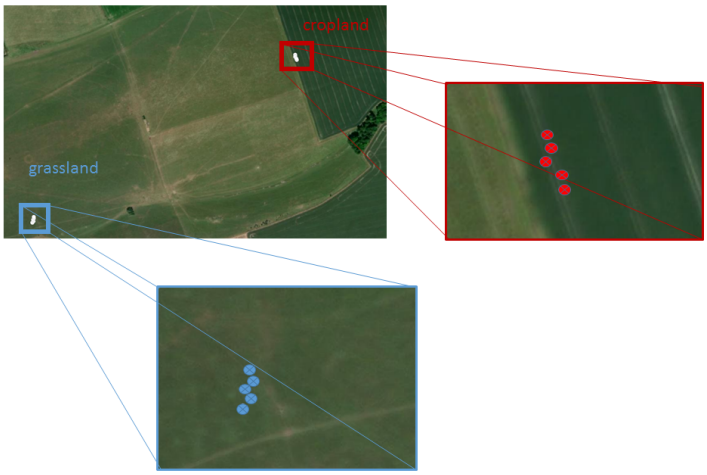
be tested, which are of importance for future generations, including pollution with new compounds like agro-chemicals and micro-plastics; as well as biodiversity or species loss to predict the resistance-resilience of soil ecosystems and their multi-functionality in relation to their specific microbial soil communities.

FIGURE A.1: A: Soil wash to to prepare the inoculum from contrasting land use, B: high intensity land use on monoculture cropland, C: adjacent pristine, species-rich grassland, D: long term bare fallow soil at Rothamsted Research, Harpenden, UK, E: overview soil mesocosm set up with wick irrigation, F: example of inoculation with soil wash from contrasting land use (+autoclaved control) in low pH treatment, G: wheat assay 12 weeks after seeding sorted from left to right according to pH treatment

Appendix A

AppendixA

(A)



(B)

FIGURE A.2: Sampling location of inoculum soil from Parsonage Down SSSI and the neighbouring field.

TABLE A.1: Co-ordinates of sites sampled for inoculation soil at Parsonage Downs.Distance between replicates: 10 m, Distance between pristine and arable land use: 1.2 km

Land use	replicate	N	W	elevation
arable	9A	51.17350	-1.91101	108
arable	8A	51.17355	-1.91187	107
arable	7A	51.17358	-1.91187	108
arable	6A	51.17362	-1.91187	107
arable	10A	51.17345	-1.91179	107
pristine	1P	51.16805	-1.9258	119
pristine	2P	51.16810	-1.92574	120
pristine	3P	51.16815	-1.92576	117
pristine	4P	51.16819	-1.92573	117
pristine	5P	51.16824	-1.92573	119

TABLE A.2: Root exudate solution applied weekly to soil mesocosms (Baudoin, Benizri, and Guckert, 2003) with C:N ratio 40.1

compound	mM	compound class	molar mass g/mol	g/l stock solution
glucose	18.4	carbohydrate	180.156	3.3149
fructose	18.4		180.16	3.3149
sucrose	9.2		342.3	3.1492
citric acid	4.6	carboxylic acid	192	0.8838
lactic acid	9.2		90.08	0.8287
succinic acid	6.9		118.09	0.8148
alanine	18.4	amino acid	89.09	1.6393
serine	18.4		105.09	1.9337
glutamic acid	11		147.13	1.6184

TABLE A.3: Functions of soil extracellular enzymes tested according to Weintraub et al. 2007 and Nyyssonen et al. 2013

enzyme		function
acetyl esterase	ACE	carbon related microbial activity
α-glucosidase	α-GLU	starch degradation
β- 1,4- glucosidase	β-GLU	short-chain cellulose oligomers especially cellobiose
N-acetyl-b-glucosaminidase	CHIN	chitin (and chitin-derived oligomers) degradation
β-1,4 -xylosidase	HEM	xylooligomer degradation to xylose
Leucine-aminopeptidase	LEU	degrades peptides to aminoacids, broad specifity
acid phosphatase	PHO	mineralisation organic P to phosphate
arylsulphatase	SUL	sulphur cycling

TABLE A.4: Pot Experiment PERMANOVA of bacterial community composition as a response of the applied treatments inoculation (land use * autoclaving) and soil pH manipulation.

PERMANOVA	**bulk soil**						**root**					
	Df	SumsOfSqs	MeanSqs	F.Mod	R2	p	Df	SumsOfSqs	MeanSqs	F.Mod	R2	p
pH												
	2	0.776	0.388	1.251	0.032	0.179	3	9.979	3.326	20.482	0.460	**0.001** ***
	75	23.247	0.310		0.968		72	11.693	0.162		0.540	
	77	24.023			1.000		75	21.672			1.000	
land use												
	3	8.249	2.750	12.898	0.343	**0.001** ***	2	0.612	0.306	1.061	0.028	0.366
	74	15.774	0.213		0.657		73	21.060	0.288		0.972	
	77	24.023			1.000		75	21.672			1.000	
autoclaving												
	2	1.738	0.869	2.925	0.072	**0.001** ***	2	1.033	0.516	1.827	0.048	0.024 *
	75	22.285	0.297		0.928		73	20.639	0.283		0.952	
	77	24.023			1.000		75	21.672			1.000	
DNA extraction	1	0.301	0.301	0.964	0.013	0.386	1	1.729	1.729	6.415	0.080	**0.001** ***
	76	23.722	0.312		0.987		74	19.943	0.270		0.920	
	77	24.023			1.000		75	21.672			1.000	

TABLE A.5: Indicator bacteria and archaea of the soil wash which was used as inoculum in a mesocosm experiment comparing contrasting land-use history effects of soil microbial communities derived from pristine, species-rich grassland soil versus arable. The 25 most abundant taxa for each land-use type are displayed which are indicator organisms according to IndVal analysis (p-value <0.05).

p-value	indval_arable	indval_pristine	mabund_arable	mabund_pristine	Phylum	Class	Order	Family	Genus	
pristine indicator bacteria										
0.017	0.04	0.94	0.0004	0.0064	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101
0.02	0.00	0.99	0.0000	0.0056	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter
0.016	0.00	0.99	0.0000	0.0055	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter
0.021	0.04	0.95	0.0003	0.0051	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Candidatus Xiphinematobacter
0.02	0.06	0.91	0.0005	0.0049	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101
0.018	0.01	0.98	0.0001	0.0047	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
0.02	0.01	0.97	0.0001	0.0047	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Candidatus Xiphinematobacter
0.023	0.12	0.88	0.0006	0.0041	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium
0.024	0.05	0.95	0.0002	0.0039	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
0.015	0.01	0.96	0.0002	0.0038	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	
0.013	0.12	0.88	0.0005	0.0037	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium
0.017	0.17	0.83	0.0007	0.0036	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
0.024	0.03	0.95	0.0002	0.0035	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101
0.037	0.19	0.81	0.0008	0.0033	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	
0.013	0.00	1.00	0.0000	0.0031	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter
0.019	0.07	0.93	0.0002	0.0030	Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	
0.009	0.10	0.85	0.0005	0.0029	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Candidatus Xiphinematobacter
0.041	0.15	0.85	0.0005	0.0029	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Phyllobacterium
0.032	0.00	1.00	0.0000	0.0027	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101
0.045	0.11	0.84	0.0005	0.0027	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Candidatus Xiphinematobacter
0.017	0.02	0.97	0.0001	0.0026	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes
0.018	0.00	1.00	0.0000	0.0026	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes
0.034	0.19	0.81	0.0006	0.0025	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter
0.01	0.00	1.00	0.0000	0.0025	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Candidatus Xiphinematobacter
0.015	0.01	0.98	0.0000	0.0025	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101
0.032	0.83	0.17	0.0153	0.0031	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium
0.031	0.83	0.17	0.0147	0.0029	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium
arable indicator bacteria										
0.013	1.00	0.00	0.0056	0.0000	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera SCA1170
0.02	1.00	0.00	0.0052	0.0000	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera SCA1171
0.031	0.98	0.01	0.0036	0.0001	Bacteria	Proteobacteria	Betaproteobacteria			
0.041	0.95	0.04	0.0034	0.0002	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.014	0.99	0.00	0.0034	0.0000	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.02	1.00	0.00	0.0032	0.0000	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera SCA1170
0.025	0.65	0.35	0.0031	0.0016	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas
0.017	0.84	0.16	0.0029	0.0006	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter
0.022	0.97	0.03	0.0029	0.0001	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.017	1.00	0.00	0.0027	0.0000	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.019	0.99	0.00	0.0027	0.0000	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.021	0.99	0.00	0.0025	0.0000	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas
0.02	0.97	0.01	0.0025	0.0001	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
0.021	0.99	0.00	0.0024	0.0000	Bacteria	Proteobacteria	Betaproteobacteria			
0.046	0.94	0.02	0.0023	0.0001	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	
0.02	0.66	0.34	0.0023	0.0012	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	
0.037	0.94	0.04	0.0022	0.0001	Bacteria	Proteobacteria	Betaproteobacteria			
0.013	0.99	0.00	0.0021	0.0000	Bacteria	Proteobacteria	Betaproteobacteria			
0.034	0.88	0.10	0.0021	0.0003	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15		
0.013	0.99	0.00	0.0021	0.0000	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas
0.012	1.00	0.00	0.0020	0.0000	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales		
0.036	0.69	0.31	0.0020	0.0009	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas
0.02	1.00	0.00	0.0020	0.0000	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	

Bibliography

Acosta-Martinez, V and MA Tabatabai (2000). "Enzyme activities in a limed agricultural soil". In: *Biology and fertility of soils* 31.1, pp. 85–91.

Allison, V. J. et al. (2007). "Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand". In: *Soil Biology and Biochemistry* 39.7, pp. 1770–1781. ISSN: 00380717. DOI: 10.1016/j.soilbio.2007.02.006.

Alyson E. Mitchell, ∗ et al. (2007). "Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes". In: *J. Agric. Food Chem.* 2007., 55, pp. ,15. DOI: 10.1021/jf070344+.

Amann, RI, W Ludwig, and KH Schleifer (1995). "Phylogenetic identification and in situ detection of individual microbial cells without cultivation". In: *Microbiol. Rev.* 59.1, pp. 143–169. URL: http://mmbr.asm.org/cgi/content/long/59/1/143.

Amaral-Zettler, Linda A. et al. (2009). "A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes". en. In: *PLOS ONE* 4.7, e6372. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0006372. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0006372 (visited on 07/26/2019).

Amelung, W. et al. (2020). "Towards a global-scale soil climate mitigation strategy". In: *Nat. Commun.* 11.5427, pp. 1–10. ISSN: 2041-1723. DOI: 10.1038/s41467-020-18887-7.

Armbruster, Melanie et al. (2020). "Bacterial and archaeal taxa are reliable indicators of soil restoration across distributed calcareous grasslands". In: *European Journal of Soil Science*.

Backer, Rachel et al. (2018). "Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture". In: *Front. Plant Sci.* 9. DOI: 10.3389/fpls.2018.01473.

Bahram, Mohammad et al. (2018). "Structure and function of the global topsoil microbiome". In: *accepted in Nature*. ISSN: 0028-0836. DOI: 10.1038/s41586-018-0386-6.

Baldrian, Petr and Rubén López-Mondéjar (2014). "Microbial genomics, transcriptomics and proteomics: new discoveries in decomposition research using complementary methods". In: *Appl. Microbiol. Biotechnol.* 98.4, pp. 1531–1537. ISSN: 1432-0614. DOI: 10.1007/s00253-013-5457-x.

Baldrian, Petr et al. (2012). "Active and total microbial communities in forest soil are largely different and highly stratified during decomposition". In: *The ISME journal* 6.2, pp. 248–258.

Banerjee, Samiran et al. (2019). "Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots". In: *ISME Journal*. ISSN: 17517370. DOI: 10.1038/s41396-019-0383-2. URL: http://dx.doi.org/10.1038/s41396-019-0383-2.

Barbabietola, Nicoletta et al. (2016). "A safe microbe-based procedure for a gentle removal of aged animal glues from ancient paper". In: *Int. Biodeterior. Biodegrad.* 109, pp. 53–60. ISSN: 0964-8305. DOI: 10.1016/j.ibiod.2015.12.019.

Bardgett, Richard D. and Tancredi Caruso (2020). "Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states". In: *Philosophical Transactions of the Royal Society B: Biological Sciences* 375.1794, p. 20190112. DOI: 10.1098/rstb.2019.0112. URL: https://royalsocietypublishing.org/doi/10.1098/rstb.2019.0112 (visited on 01/27/2020).

Bardgett, Richard D, Chris Freeman, and Nicholas J Ostle (2008). "Microbial contributions to climate change through carbon cycle feedbacks". In: *The ISME journal* 2.8, pp. 805–814.

Bardgett, Richard D., Philip J. Hobbs, and Åsa Frostegård (1996). "Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland". In: *Biol. Fertil. Soils* 22.3, pp. 261–264. ISSN: 1432-0789. DOI: 10.1007/BF00382522.

Baron, Noemi Carla, Andressa de Souza Pollo, and Everlon Cid Rigobelo (2020). "Purpureocillium lilacinum and Metarhizium marquandii as plant growth-promoting fungi". In: *PeerJ* 8. DOI: 10.7717/peerj.9005.

Bates, Douglas et al. (2014). "Fitting Linear Mixed-Effects Models using lme4". In: 67.1. DOI: 10.18637/jss.v067.i01. URL: http://arxiv.org/abs/1406.5823.

Baudoin, Ezékiel, Emile Benizri, and Armand Guckert (2003). "Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere". In: *Soil Biology and Biochemistry* 35.9, pp. 1183–1192. ISSN: 00380717. DOI: 10.1016/S0038-0717(03)00179-2.

Bell, Thomas et al. (2005). "The contribution of species richness and composition to bacterial services". In: *Nature* 436.7054, pp. 1157–1160. ISSN: 1476-4687. DOI: 10.1038/nature03891.

Bender, S. Franz, Cameron Wagg, and Marcel G. A. van der Heijden (2016). "An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability". en. In: *Trends in Ecology & Evolution* 31.6, pp. 440–452. ISSN: 0169-5347. DOI: 10.1016/j.tree.2016.02.016. URL: http://www.sciencedirect.com/science/article/pii/S0169534716000616 (visited on 01/07/2020).

Bengtsson, Jan et al. (2000). "Biodiversity, disturbances, ecosystem function and management of European forests". In: *Forest ecology and management* 132.1, pp. 39–50.

Berg, Ivan A. et al. (2010). "Autotrophic carbon fixation in archaea". en. In: *Nature Reviews Microbiology* 8.6, pp. 447–460. ISSN: 1740-1526, 1740-1534. DOI: 10.1038/nrmicro2365. URL: http://www.nature.com/articles/nrmicro2365 (visited on 11/12/2019).

Bergmann, Gaddy T. et al. (2011). "The under-recognized dominance of Verrucomicrobia in soil bacterial communities". In: *Soil biology & biochemistry* 43.7, pp. 1450–

1455. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2011.03.012. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3260529/ (visited on 10/27/2019).

Berry, David and Stefanie Widder (2014). "Deciphering microbial interactions and detecting keystone species with co-occurrence networks". In: *Front. Microbiol.* 5. ISSN: 1664-302X. DOI: 10.3389/fmicb.2014.00219.

Bevan, Rachel B. and B. Franz Lang (2004). "Mitochondrial genome evolution: the origin of mitochondria and of eukaryotes". en. In: *Mitochondrial Function and Biogenesis*. Topics in Current Genetics. Berlin, Heidelberg: Springer, pp. 1–35. ISBN: 978-3-540-40895-6. DOI: 10.1007/b96830. URL: https://doi.org/10.1007/b96830 (visited on 11/20/2019).

Biodiversity, Intergovernmental Science-Policy Platform on and Ipbes. Ecosystem Services (2018). "The IPBES assessment report on land degradation and restoration." In: *Zenodo*. DOI: 10.5281/zenodo.3237393.

Biodiversity, Intergovernmental Science-Policy Platform on and Ipbes Ecosystem Services (2019). "Summary for policymakers of the global assessment report on biodiversity and ecosystem services". In: *Zenodo*. DOI: 10.5281/zenodo.3553579.

Boddy, Lynne (2016). "Pathogens of Autotrophs". In: *The Fungi: Third Edition*, pp. 245–292. ISBN: 978-0-12-382034-1. DOI: 10.1016/B978-0-12-382034-1.00008-6.

Bond-Lamberty, Ben and Allison Thomson (2010). "Temperature-associated increases in the global soil respiration record". In: *Nature* 464, pp. 579–582. ISSN: 1476-4687. DOI: 10.1038/nature08930.

Bongiorno, Giulia et al. (2019). "Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe". In: *Ecological Indicators* 99.September 2018, pp. 38–50. ISSN: 1470160X. DOI: 10.1016/j.ecolind.2018.12.008. URL: https://linkinghub.elsevier.com/retrieve/pii/S1470160X18309415.

Bouchez, T. et al. (2016). "Molecular microbiology methods for environmental diagnosis". In: *Environmental Chemistry Letters* 14.4, pp. 423–441. ISSN: 16103661. DOI: 10.1007/s10311-016-0581-3.

Brenner, Katie, Lingchong You, and Frances H. Arnold (2008). "Engineering microbial consortia: a new frontier in synthetic biology". In: *Trends Biotechnol.* 26.9, pp. 483–489. ISSN: 0167-7799. DOI: 10.1016/j.tibtech.2008.05.004.

Bressan, Mélanie et al. (2015). "A rapid flow cytometry method to assess bacterial abundance in agricultural soil". en. In: *Applied Soil Ecology* 88, pp. 60–68. ISSN: 0929-1393. DOI: 10.1016/j.apsoil.2014.12.007. URL: http://www.sciencedirect.com/science/article/pii/S0929139314003503 (visited on 11/25/2019).

Brewer, Tess E. et al. (2017). "Genome reduction in an abundant and ubiquitous soil bacterium 'Candidatus Udaeobacter copiosus'". en. In: *Nature Microbiology* 2.2. ISSN: 2058-5276. DOI: 10.1038/nmicrobiol.2016.198. URL: http://www.nature.com/articles/nmicrobiol2016198 (visited on 07/08/2019).

Bronick, C. J. and R. Lal (2005). *Soil structure and management: A review*. Vol. 124. 1-2. ISBN: 0016-7061. DOI: 10.1016/j.geoderma.2004.03.005.

Bullock, James M. (2011). *UK National Ecosystem Assessment: Technical Report Chapter 6*. Tech. rep.

Bulman, Simon et al. (2007). "Intron-rich Gene Structure in the Intracellular Plant Parasite Plasmodiophora brassicae". In: *Protist* 158.4, pp. 423–433. ISSN: 1434-4610. DOI: 10.1016/j.protis.2007.04.005.

Burns, Richard G. et al. (2013). "Soil enzymes in a changing environment: Current knowledge and future directions". In: *Soil Biology and Biochemistry* 58, pp. 216–234. ISSN: 00380717. DOI: 10.1016/j.soilbio.2012.11.009. URL: http://dx.doi.org/10.1016/j.soilbio.2012.11.009.

Callahan, Benjamin J et al. (2016). "DADA2: High-resolution sample inference from Illumina amplicon data". In: *Nature Methods* 13, p. 581. URL: http://dx.doi.org/10.1038/nmeth.3869http://10.0.4.14/nmeth.3869https://www.nature.com/articles/nmeth.3869#supplementary-information.

Carini, Paul et al. (2016). "Relic DNA is abundant in soil and obscures estimates of soil microbial diversity". In: *bioRxiv*, p. 043372. DOI: 10.1101/043372. eprint: 043372.

Cerecetto, Victoria et al. (2021). "Reduced tillage, cover crops and organic amendments affect soil microbiota and improve soil health in Uruguayan vegetable farming systems". In: *FEMS Microbiology Ecology* 97.3, fiab023.

Chase, Jonathan M. et al. (2020). "Ecosystem decay exacerbates biodiversity loss with habitat loss". In: *Nature*, pp. 1–6. ISSN: 1476-4687. DOI: 10.1038/s41586-020-2531-2.

Ciancio, Aurelio, Corné MJ Pieterse, and Jesús Mercado-Blanco (2016). "Harnessing useful rhizosphere microorganisms for pathogen and pest biocontrol". In: *Frontiers in microbiology* 7, p. 1620.

Cortois, Roeland and Gerlinde Barbara De Deyn (2012). "The curse of the black box". In: *Plant and Soil* 350.1-2, pp. 27–33.

Costanza, Robert et al. (1997). "The value of the world's ecosystem services and natural capital". In: *Nature* 387.6630, pp. 253–260. ISSN: 1476-4687. DOI: 10.1038/387253a0.

Cotrufo, M. Francesca et al. (2013). "The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter?" In: *Global Change Biology* 19.4, pp. 988–995. ISSN: 13541013. DOI: 10.1111/gcb.12113.

Cotrufo, M. Francesca et al. (2015). "Formation of soil organic matter via biochemical and physical pathways of litter mass loss". In: *Nature Geoscience* 8.10, pp. 776–779. ISSN: 17520908. DOI: 10.1038/ngeo2520.

Coyle, Cait et al. (2016). "A Functional Land Management conceptual framework under soil drainage and land use scenarios". In: *Environmental Science and Policy* 56, pp. 39–48. ISSN: 18736416. DOI: 10.1016/j.envsci.2015.10.012. URL: http://dx.doi.org/10.1016/j.envsci.2015.10.012.

Crowther, T W, C Averill, and D S Maynard (2019). "The global soil community and its influence on biogeochemistry". en. In: *SOIL ECOLOGY*, p. 12.

Csardi, G and T Nepusz (2006). "The igraph software package for complex network research". In: Complex Systems, p. 1695. URL: http://igraph.org.

Cullen, DW and PR Hirsch (1998). "Simple and rapid method fordirect extraction of microbial DNA fromsoil for PCR". In: *Soil Biology and Biochemistry* 30.8-9, pp. 983–993.

Daily, Gretchen C et al. (1997). *Nature's services*. Vol. 3. Island Press, Washington, DC.

De Bruyn, LA Lobry (1999). "Ants as bioindicators of soil function in rural environments". In: *Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes*. Elsevier, pp. 425–441.

De Roy, Karen et al. (2013). "Environmental conditions and community evenness determine the outcome of biological invasion". In: *Nature communications* 4.1, pp. 1–5.

Delgado-Baquerizo, Manuel et al. (2018). "A global atlas of the dominant bacteria found in soil". In: *Science* 359.6373, pp. 320–325.

Delgado-Baquerizo, Manuel et al. (2020). "Multiple elements of soil biodiversity drive ecosystem functions across biomes". en. In: *Nature Ecology & Evolution* 4.2, pp. 210–220. ISSN: 2397-334X. DOI: 10.1038/s41559-019-1084-y. URL: https://www.nature.com/articles/s41559-019-1084-y (visited on 02/07/2020).

Deng, Lei et al. (2016). "Global patterns of the effects of land-use changes on soil carbon stocks". en. In: *Global Ecology and Conservation* 5, pp. 127–138. ISSN: 2351-9894. DOI: 10.1016/j.gecco.2015.12.004. URL: http://www.sciencedirect.com/science/article/pii/S2351989415300226 (visited on 11/08/2019).

Dennis, Paul G., Anthony J. Miller, and Penny R. Hirsch (2010). "Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?: Root exudates and rhizosphere bacteria". en. In: *FEMS Microbiology Ecology* 72.3, pp. 313–327. ISSN: 01686496, 15746941. DOI: 10.1111/j.1574-6941.2010.00860.x. URL: https://academic.oup.com/femsec/article-lookup/doi/10.1111/j.1574-6941.2010.00860.x (visited on 08/29/2019).

Dequiedt, Samuel et al. (2009). "Biogeographical patterns of soil bacterial communities". In: *Environmental Microbiology Reports* 1.4, pp. 251–255. ISSN: 1758-2229. DOI: 10.1111/j.1758-2229.2009.00040.x.

DeSantis, T. Z. et al. (2006). "Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB". eng. In: *Applied and Environmental Microbiology* 72.7, pp. 5069–5072. ISSN: 0099-2240. DOI: 10.1128/AEM.03006-05.

Detheridge, Andrew P. et al. (2016). "The legacy effect of cover crops on soil fungal populations in a cereal rotation". en. In: *Agriculture, Ecosystems & Environment* 228, pp. 49–61. ISSN: 0167-8809. DOI: 10.1016/j.agee.2016.04.022. URL: http://www.sciencedirect.com/science/article/pii/S0167880916302250 (visited on 11/29/2019).

Didion, Markus et al. (2016). "Towards harmonizing leaf litter decomposition studies using standard tea bags-a field study and model application". In: *Forests* 7.8, pp. 1–12. ISSN: 19994907. DOI: 10.3390/f7080167.

Dinolfo, María Inés, Eliana Castañares, and Sebastian Alberto Stenglein (2017). "Fusarium-plant interaction: state of the art-a review". In:

Dominati, Estelle, Murray Patterson, and Alec Mackay (2010). "A framework for classifying and quantifying the natural capital and ecosystem services of soils". In: *Ecol. Econ.* 69.9, pp. 1858–1868. ISSN: 0921-8009. DOI: 10.1016/j.ecolecon.2010.05.002.

Don, Axel et al. (2017). "Microbial community composition affects soil organic carbon turnover in mineral soils". In: *Biology and Fertility of Soils* 53.4, pp. 445–456. ISSN: 01782762. DOI: 10.1007/s00374-017-1198-9.

Dufrene, Marc et al. (2011). "Species Assemblages and Indicator Species : The Need for a Flexible Asymmetrical Approach SPECIES ASSEMBLAGES AND INDICATOR SPECIES : THE NEED FOR A FLEXIBLE ASYMMETRICAL APPROACH". In: *America* 67.3, pp. 345–366.

Ecclestone, P (2001). "Minimal tillage options for economic sugar beet production". In: *British Sugar Beet Review* 69.3, pp. 24–29.

Egidi, Eleonora et al. (2019). "A few Ascomycota taxa dominate soil fungal communities worldwide". In: *Nat. Commun.* 10.2369, pp. 1–9. ISSN: 2041-1723. DOI: 10.1038/s41467-019-10373-z.

Elsas, Jan Dirk van et al. (2012). "Microbial diversity determines the invasion of soil by a bacterial pathogen". In: *Proc. Natl. Acad. Sci. U.S.A.* 109.4, pp. 1159–1164. ISSN: 0027-8424. DOI: 10.1073/pnas.1109326109.

Emmerling, C., T. Udelhoven, and D. Schröder (2001). "Response of soil microbial biomass and activity to agricultural de-intensification over a 10year period". en. In: *Soil Biology and Biochemistry* 33.15, pp. 2105–2114. ISSN: 0038-0717. DOI: 10.1016/S0038-0717(01)00143-2. URL: http://www.sciencedirect.com/science/article/pii/S0038071701001432 (visited on 11/27/2019).

Erisman, Jan Willem et al. (2008). "How a century of ammonia synthesis changed the world". In: *Nat. Geosci.* 1, pp. 636–639. ISSN: 1752-0908. DOI: 10.1038/ngeo325.

Eyre, Michael D., Derek A. Lott, and Adam Garside (1996). "Assessing the potential for environmental monitoring using ground beetles (Coleoptera: Carabidae) with riverside and Scottish data". In: *Ann. Zool. Fenn.* 33.1, pp. 157–163. ISSN: 0003-455X. URL: https://www.jstor.org/stable/23735415.

Fagan, Kate C. et al. (2008). "Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes". In: *Journal of Applied Ecology* 45.4, pp. 1293–1303. ISSN: 00218901. DOI: 10.1111/j.1365-2664.2008.01492.x.

— (2010). "The seed banks of English lowland calcareous grasslands along a restoration chronosequence". In: *Plant Ecology* 208.2, pp. 199–211. ISSN: 13850237. DOI: 10.1007/s11258-009-9698-9.

Fahner, Nicole A. et al. (2016). "Large-scale monitoring of plants through environmental DNA metabarcoding of soil: Recovery, resolution, and annotation of four DNA markers". In: *PLoS ONE* 11.6, pp. 1–16. ISSN: 19326203. DOI: 10.1371/journal.pone.0157505.

FAO and ITPS (2015). *The Status of the World's Soil Resources (Main Report)*. ISBN: 978-92-5-109004-6. URL: https://hal.archives-ouvertes.fr/hal-01241064/.

FAO et al. (2019). *The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. Rome, FAO.*

Fierer, Noah (2017). "Embracing the unknown: disentangling the complexities of the soil microbiome". In: *Nature Reviews Microbiology*. ISSN: 1740-1526. DOI: 10.1038/nrmicro.2017.87. URL: http://www.nature.com/doifinder/10.1038/nrmicro.2017.87.

Fierer, Noah, Mark A. Bradford, and Robert B. Jackson (2007). "Toward an ecological classification of soil bacteria". In: *Ecology* 88.6, pp. 1354–1364. ISSN: 00129658. DOI: 10.1890/05-1839.

Fierer, Noah and Robert B. Jackson (2006). "The diversity and biogeography of soil bacterial communities". In: *Proc. Natl. Acad. Sci. U.S.A.* 103.3, pp. 626–631. ISSN: 0027-8424. DOI: 10.1073/pnas.0507535103.

Fierer, Noah, Stephen A. Wood, and Clifton P. Bueno de Mesquita (2020). "How microbes can, and cannot, be used to assess soil health". In: *EcoEvoRxiv*. DOI: 10.32942/osf.io/t9d5y.

Fiore-Donno, Anna Maria et al. (2019). "Functional Traits and Spatio-Temporal Structure of a Major Group of Soil Protists (Rhizaria: Cercozoa) in a Temperate Grassland". In: *Front. Microbiol.* 10. ISSN: 1664-302X. DOI: 10.3389/fmicb.2019.01332.

Foley, Jonathan A. et al. (2005). "Global Consequences of Land Use". In: *Science* 309.5734, pp. 570–574. ISSN: 0036-8075. DOI: 10.1126/science.1111772.

French, Katherine E., Andrzej Tkacz, and Lindsay A. Turnbull (2017). "Conversion of grassland to arable decreases microbial diversity and alters community composition". In: *Applied Soil Ecology* 110, pp. 43–52. ISSN: 0929-1393. DOI: 10.1016/j.apsoil.2016.10.015. URL: http://www.sciencedirect.com/science/article/pii/S0929139316305248 (visited on 07/18/2019).

Geisen, Stefan et al. (2019). "A methodological framework to embrace soil biodiversity". In: *Soil Biol. Biochem.* 136, p. 107536. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2019.107536.

George, Paul B. L. et al. (2019). "Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems". In: *Nat. Commun.* 10.1107, pp. 1–11. ISSN: 2041-1723. DOI: 10.1038/s41467-019-09031-1.

Gilbert, Jack A., Janet K. Jansson, and Rob Knight (2014). "The Earth Microbiome project: successes and aspirations". In: *BMC Biol.* 12.1, pp. 69–4. ISSN: 1741-7007. DOI: 10.1186/s12915-014-0069-1.

Gkarmiri, Konstantia et al. (2017). "Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape". In: *Applied and environmental microbiology* 83.22, e01938–17.

Glick, Bernard R (1995). "The enhancement of plant growth by free-living bacteria". In: *Canadian journal of microbiology* 41.2, pp. 109–117.

— (2010). "Using soil bacteria to facilitate phytoremediation". In: *Biotechnology advances* 28.3, pp. 367–374.

— (2014). "Bacteria with ACC deaminase can promote plant growth and help to feed the world". In: *Microbiological research* 169.1, pp. 30–39.

Godfray, H. C. J. et al. (2011). "Linking Policy on Climate and Food". In: *Science* 331.6020, pp. 1013–1014. ISSN: 0036-8075. DOI: 10.1126/science.1202899.

Golzar, H. et al. (2019). "Neoascochyta species cause leaf scorch on wheat in Australia". en. In: *Australasian Plant Disease Notes* 14.1, p. 1. ISSN: 1833-928X. DOI: 10.1007/s13314-018-0332-3. URL: https://doi.org/10.1007/s13314-018-0332-3 (visited on 07/08/2019).

Griffiths, B. S. et al. (2001). "An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities". In: *Soil Biology and Biochemistry* 33.12-13, pp. 1713–1722. ISSN: 00380717. DOI: 10.1016/S0038-0717(01)00094-3.

Griffiths, B. S. et al. (2004). "The Relationship between Microbial Community Structure and Functional Stability, Tested Experimentally in an Upland Pasture Soil". In: *Microb. Ecol.* 47.1, pp. 104–113. ISSN: 1432-184X. DOI: 10.1007/s00248-002-2043-7.

Griffiths, B. S. et al. (2016a). "Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function". In: *Ecol. Indic.* 69, pp. 213–223. ISSN: 1470-160X. DOI: 10.1016/j.ecolind.2016.04.023.

Griffiths, B. S. et al. (2016b). "Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function". In: *Ecological Indicators* 69.October, pp. 213–223. ISSN: 1470160X. DOI: 10.1016/j.ecolind.2016.04.023. URL: http://dx.doi.org/10.1016/j.ecolind.2016.04.023.

Griffiths, Bryan S. and Laurent Philippot (2013). "Insights into the resistance and resilience of the soil microbial community". en. In: *FEMS Microbiology Reviews* 37.2, pp. 112–129. ISSN: 0168-6445. DOI: 10.1111/j.1574-6976.2012.00343.x.

URL: https://academic.oup.com/femsre/article/37/2/112/622548 (visited on 11/21/2019).

Griffiths, Robert I et al. (2000). "Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA-and rRNA-based microbial community composition". In: *Applied and environmental microbiology* 66.12, pp. 5488–5491.

Griffiths, Robert I. et al. (2011). "The bacterial biogeography of British soils". In: *Environmental Microbiology* 13.6, pp. 1642–1654. ISSN: 14622912. DOI: 10.1111/j.1462-2920.2011.02480.x.

Griffiths, Robert I. et al. (2016c). "Mapping and validating predictions of soil bacterial biodiversity using European and national scale datasets". In: *Applied Soil Ecology* 97, pp. 61–68. ISSN: 09291393. DOI: 10.1016/j.apsoil.2015.06.018.

Grime, J. P. (1997). "Biodiversity and Ecosystem Function: The Debate Deepens". In: *Science* 277.5330, pp. 1260–1261. ISSN: 0036-8075. DOI: 10.1126/science.277.5330.1260.

Großkopf, Tobias and Orkun S. Soyer (2014). "Synthetic microbial communities". In: *Curr. Opin. Microbiol.* 18, pp. 72–77. ISSN: 1369-5274. DOI: 10.1016/j.mib.2014.02.002.

Gu, Zuguang et al. (2014). "Circlize implements and enhances circular visualization in R". In: *Bioinformatics* 30.19, pp. 2811–2812. ISSN: 14602059. DOI: 10.1093/bioinformatics/btu393.

Guerra, Carlos A. et al. (2020). "Blind spots in global soil biodiversity and ecosystem function research". In: *Nat. Commun.* 11.3870, pp. 1–13. ISSN: 2041-1723. DOI: 10.1038/s41467-020-17688-2.

Guo, Yuan et al. (2018). "Effects of Microbial Induced Calcite Precipitation on Bentonite Cracking Remediation". In: *American Society of Civil Engineers*, pp. 135–144. DOI: 10.1061/9780784481592.014.

Haddaway, Neal R. et al. (2017). "How does tillage intensity affect soil organic carbon? A systematic review". In: *Environ. Evid.* 6.1, pp. 1–48. ISSN: 2047-2382. DOI: 10.1186/s13750-017-0108-9.

Hadley Wickham (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag. ISBN: 978-3-319-24277-4.

Harman, Gary E. et al. (2004). "Trichoderma species — opportunistic, avirulent plant symbionts". In: *Nat. Rev. Microbiol.* 2, pp. 43–56. ISSN: 1740-1534. DOI: 10.1038/nrmicro797.

Hartman, Kyle et al. (2018). "Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming". en. In: *Microbiome* 6.1. ISSN: 2049-2618. DOI: 10.1186/s40168-017-0389-9. URL: https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-017-0389-9 (visited on 09/04/2019).

Hartman, Wyatt H et al. (2017). "A genomic perspective on stoichiometric regulation of soil carbon cycling". In: *The ISME Journal*, pp. 1–14. ISSN: 1751-7362. DOI: 10.1038/ismej.2017.115. URL: http://www.nature.com/doifinder/10.1038/ismej.2017.115.

Hättenschwiler, Stephan, Alexei V Tiunov, and Stefan Scheu (2005). "Biodiversity and litter decomposition in terrestrial ecosystems". In: *Annu. Rev. Ecol. Evol. Syst.* 36, pp. 191–218.

Hayden, Helen L. et al. (2010). "The abundance of nitrogen cycle genes amoA and nifH depends on land-uses and soil types in South-Eastern Australia". In: *Soil Biol. Biochem.* 42.10, pp. 1774–1783. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2010.06.015.

Hebert, Paul DN et al. (2003). "Biological identifications through DNA barcodes". In: *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270.1512, pp. 313–321.

Helander, Marjo et al. (2018). "Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback". In: *Sci. Total Environ.* 642, pp. 285–291. ISSN: 0048-9697. DOI: 10.1016/j.scitotenv.2018.05.377.

Hermans, Syrie M et al. (2017). "Bacteria as emerging indicators of soil condition". In: *Appl. Environ. Microbiol.* 83.1, e02826–16.

Hermans, Syrie M. et al. (2020). "Using soil bacterial communities to predict physico-chemical variables and soil quality". In: *Microbiome* 8.1, pp. 1–13. ISSN: 2049-2618. DOI: 10.1186/s40168-020-00858-1.

Hirsch, Penny R., Tim H. Mauchline, and Ian M. Clark (2010). "Culture-independent molecular techniques for soil microbial ecology". In: *Soil Biology and Biochemistry*

42.6, pp. 878–887. ISSN: 00380717. DOI: 10.1016/j.soilbio.2010.02.019. URL: http://dx.doi.org/10.1016/j.soilbio.2010.02.019.

Hirsch, Penny R. et al. (2009). "Starving the soil of plant inputs for 50 years reduces abundance but not diversity of soil bacterial communities". In: *Soil Biology and Biochemistry* 41.9, pp. 2021–2024. ISSN: 00380717. DOI: 10.1016/j.soilbio.2009.07.011. URL: http://linkinghub.elsevier.com/retrieve/pii/S0038071709002569.

Hirsch, Penny R. et al. (2016). *Soil resilience and recovery: rapid community responses to management changes*. DOI: 10.1007/s11104-016-3068-x.

Howard, Mia M., Terrence H. Bell, and Jenny Kao-Kniffin (2017). "Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment". In: *FEMS Microbiology Letters* 364.11, pp. 1–8. ISSN: 15746968. DOI: 10.1093/femsle/fnx092.

Hu, Jie et al. (2016). "Probiotic Diversity Enhances Rhizosphere Microbiome Function and Plant Disease Suppression". In: *mBio* 7.6. ISSN: 2150-7511. DOI: 10.1128/mBio.01790-16.

Hu, Lingfei et al. (2018). "Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota". In: *Nature Communications* 9.1, p. 2738. ISSN: 2041-1723. DOI: 10.1038/s41467-018-05122-7. URL: http://www.nature.com/articles/s41467-018-05122-7.

Huber, Katharina J. et al. (2016). "The first representative of the globally widespread subdivision 6 Acidobacteria,Vicinamibacter silvestris gen. nov., sp. nov., isolated from subtropical savannah soil". In: *Int. J. Syst. Evol. Microbiol.* 66.8, pp. 2971–2979. ISSN: 1466-5026. DOI: 10.1099/ijsem.0.001131.

Huston, Michael A. (1997). "Hidden Treatments in Ecological Experiments: Re-Evaluating the Ecosystem Function of Biodiversity". In: *Oecologia* 110.4, pp. 449–460. ISSN: 0029-8549. URL: https://www.jstor.org/stable/4221630.

Ihrmark, Katarina et al. (2012). "New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities". In: *FEMS Microbiology Ecology* 82.3, pp. 666–677. ISSN: 0168-6496. DOI: 10.1111/j.1574-6941.2012.01437.x. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1574-6941.2012.01437.x (visited on 07/26/2019).

Jenkinson, D. S. and D. S. Powlson (1976). "The effects of biocidal treatments on metabolism in soil—V: A method for measuring soil biomass". In: *Soil Biol. Biochem.* 8.3, pp. 209–213. ISSN: 0038-0717. DOI: 10.1016/0038-0717(76)90005-5.

Jones, Briony A. et al. (2019a). "Beyond taxonomic identification: integration of ecological responses to a soil bacterial 16S rRNA gene database". In: *bioRxiv*, p. 843847. eprint: 843847. URL: https://doi.org/10.1101/843847.

Jones, Davey L. et al. (2019b). "pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities". en. In: *Soil Biology and Biochemistry* 138, p. 107584. ISSN: 00380717. DOI: 10.1016/j.soilbio.2019.107584. URL: https://linkinghub.elsevier.com/retrieve/pii/S0038071719302482 (visited on 09/25/2019).

Jones, David L. and Eva Oburger (2010). "Solubilization of Phosphorus by Soil Microorganisms". In: *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*. Springer, Berlin, Heidelberg, pp. 169–198. ISBN: 978-3-642-15270-2. DOI: 10.1007/978-3-642-15271-9_7.

Jones, Frances Patricia et al. (2016). "Novel European free-living, non-diazotrophic Bradyrhizobium isolates from contrasting soils that lack nodulation and nitrogen fixation genes – a genome comparison". en. In: *Scientific Reports* 6.1, pp. 1–10. ISSN: 2045-2322. DOI: 10.1038/srep25858. URL: https://www.nature.com/articles/srep25858 (visited on 12/02/2019).

Jäschke, D. et al. (2010). "Suppression of clubroot (Plasmodiophora brassicae) development in Arabidopsis thaliana by the endophytic fungus Acremonium alternatum". In: *Plant Pathology* 59.1, pp. 100–111. ISSN: 00320862. DOI: 10.1111/j.1365-3059.2009.02199.x. URL: http://doi.wiley.com/10.1111/j.1365-3059.2009.02199.x.

Kaiser, Kristin et al. (2016). "Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests". In: *Scientific Reports* 6.1, p. 33696. ISSN: 2045-2322. DOI: 10.1038/srep33696. URL: http://www.nature.com/articles/srep33696.

Kalisky, Tomer and Stephen R. Quake (2011). "Single-cell genomics". In: *Nat. Methods* 8, pp. 311–314. ISSN: 1548-7105. DOI: 10.1038/nmeth0411-311.

Kallenbach, C. M., AS Grandy, and S. D. Frey (2016). "Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls". In: *Nature Communications* in revisio, pp. 1–10. ISSN: 2041-1723. DOI: 10.1038/ncomms13630. URL: http://dx.doi.org/10.1038/ncomms13630.

Karlen, Douglas L. et al. (2019). "Soil health assessment: Past accomplishments, current activities, and future opportunities". In: *Soil Tillage Res.* 195, p. 104365. ISSN: 0167-1987. DOI: 10.1016/j.still.2019.104365.

Karlík, Petr and Peter Poschlod (2019). "Identifying plant and environmental indicators of ancient and recent calcareous grasslands". In: *Ecological Indicators* 104.March, pp. 405–421. ISSN: 1470160X. DOI: 10.1016/j.ecolind.2019.05.016. URL: https://doi.org/10.1016/j.ecolind.2019.05.016.

Keuskamp, Joost A. et al. (2013). "Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems". In: *Methods Ecol. Evol.* 4.11, pp. 1070–1075. ISSN: 2041-210X. DOI: 10.1111/2041-210X.12097.

Kibblewhite, M G., K. Ritz, and M J. Swift (2007). "Soil health in agricultural systems". In: *Phil. Trans. R. Soc. B* 363.1492, pp. 685–701. ISSN: 1471-2970. URL: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2007.2178.

Kim, Nakian et al. (2020). "Do cover crops benefit soil microbiome? A meta-analysis of current research". en. In: *Soil Biology and Biochemistry* 142, p. 107701. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2019.107701. URL: http://www.sciencedirect.com/science/article/pii/S0038071719303657 (visited on 01/22/2020).

King, John D., David C. White, and Cecile W. Taylor (1977). "Use of Lipid Composition and Metabolism to Examine Structure and Activity of Estuarine Detrital Microflora". In: *Appl. Environ. Microbiol.* 33.5, pp. 1177–1183. ISSN: 0099-2240. URL: https://aem.asm.org/content/33/5/1177.short.

Koljalg, U. et al. (2005). "UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi". eng. In: *New Phytologist* 166.3, pp. 1063–1068. ISSN: 1469-8137. URL: http://lup.lub.lu.se/record/146804 (visited on 09/30/2019).

Kozich, James J. et al. (2013). "Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform". In: *Applied and Environmental Microbiology* 79.17, pp. 5112–5120. ISSN: 00992240. DOI: 10.1128/AEM.01043-13.

Koziol, Liz and James D. Bever (2016). "The missing link in grassland restoration: Arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession". In: *Journal of Applied Ecology*. ISSN: 13652664. DOI: 10.1111/1365-2664.12843.

Kuo, S. and U. M. Sainju (1998). "Nitrogen mineralization and availability of mixed leguminous and non-leguminous cover crop residues in soil". In: *Biol. Fertil. Soils* 26.4, pp. 346–353. ISSN: 1432-0789. DOI: 10.1007/s003740050387.

La Notte, Alessandra et al. (2017a). "Ecosystem services classification: A systems ecology perspective of the cascade framework". In: *Ecol. Indic.* 74, pp. 392–402. ISSN: 1470-160X. DOI: 10.1016/j.ecolind.2016.11.030.

La Notte, Alessandra et al. (2017b). "Physical and monetary ecosystem service accounts for Europe: A case study for in-stream nitrogen retention". In: *Ecosystem services* 23, pp. 18–29.

Lal, R. (2004a). "Soil carbon sequestration to mitigate climate change". In: *Geoderma* 123.1, pp. 1–22. ISSN: 0016-7061. DOI: 10.1016/j.geoderma.2004.01.032.

Lal, Rattan (2004b). "Soil carbon sequestration impacts on global climate change and food security." In: *Science (New York, N.Y.)* 304.5677, pp. 1623–7. ISSN: 1095-9203. DOI: 10.1126/science.1097396. URL: http://www.ncbi.nlm.nih.gov/pubmed/15192216.

— (2019). "Managing Soils for Resolving the Conflict Between Agriculture and Nature: The Hard Talk". en. In: *European Journal of Soil Science*. ISSN: 13510754. DOI: 10.1111/ejss.12857. URL: http://doi.wiley.com/10.1111/ejss.12857 (visited on 07/04/2019).

Lange, Markus et al. (2015). "Plant diversity increases soil microbial activity and soil carbon storage." In: *Nature communications* 6, p. 6707. ISSN: 2041-1723. DOI: 10.1038/ncomms7707. URL: http://www.ncbi.nlm.nih.gov/pubmed/25848862.

Lanzén, Anders et al. (2016). "Multi-targeted metagenetic analysis of the influence of climate and environmental parameters on soil microbial communities along

an elevational gradient". In: *Sci. Rep.* 6.28257, pp. 1–13. ISSN: 2045-2322. DOI: 10.1038/srep28257.

Lanzén, Anders et al. (2015). "The Community Structures of Prokaryotes and Fungi in Mountain Pasture Soils are Highly Correlated and Primarily Influenced by pH". English. In: *Frontiers in Microbiology* 6. ISSN: 1664-302X. DOI: 10.3389/fmicb.2015.01321. URL: https://www.frontiersin.org/articles/10.3389/fmicb.2015.01321/full (visited on 11/25/2019).

Lauber, Christian L. et al. (2008). "The influence of soil properties on the structure of bacterial and fungal communities across land-use types". en. In: *Soil Biology and Biochemistry*. Special Section: Enzymes in the Environment 40.9, pp. 2407–2415. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2008.05.021. URL: http://www.sciencedirect.com/science/article/pii/S0038071708001764 (visited on 11/27/2019).

Lauber, Christian L. et al. (2009). "Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale". In: *Appl. Environ. Microbiol.* 75.15, pp. 5111–5120. ISSN: 0099-2240. DOI: 10.1128/AEM.00335-09.

Le Bagousse-Pinguet, Yoann et al. (2019). "Phylogenetic, functional, and taxonomic richness have both positive and negative effects on ecosystem multifunctionality". In: *Proc. Natl. Acad. Sci. U.S.A.* 116.17, pp. 8419–8424. ISSN: 0027-8424. DOI: 10.1073/pnas.1815727116.

Lehmann, Johannes et al. (2020). "Persistence of soil organic carbon caused by functional complexity". In: *Nat. Geosci.* 13.8, pp. 529–534. ISSN: 1752-0908. DOI: 10.1038/s41561-020-0612-3.

Li, Fang et al. (2018). "Mortierella elongata's roles in organic agriculture and crop growth promotion in a mineral soil". en. In: *Land Degradation & Development* 29.6, pp. 1642–1651. ISSN: 1099-145X. DOI: 10.1002/ldr.2965. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/ldr.2965 (visited on 11/28/2019).

LI, Yujuan et al. (2005). "Nematodes as bioindicator of soil health: methods and applications". In: *Chinese Journal of Applied Ecology* 8, p. 30.

Liddicoat, Craig et al. (2019). "Can bacterial indicators of a grassy woodland restoration inform ecosystem assessment and microbiota-mediated human health?" en. In: *Environment International* 129, pp. 105–117. ISSN: 0160-4120. DOI: 10.1016/j.envint.2019.05.011. URL: http://www.sciencedirect.com/science/article/pii/S0160412018331386 (visited on 10/27/2019).

Liu, Hongwei et al. (2018). "Activation of the salicylic acid signalling pathway in wheat had no significant short-term impact on the diversity of root-associated microbiomes". In: *Pedobiologia* 70.August, pp. 6–11. ISSN: 00314056. DOI: 10.1016/j.pedobi.2018.08.001. URL: https://doi.org/10.1016/j.pedobi.2018.08.001.

Lu, Huilin et al. (2020). "Alkaline amendments improve the health of soils degraded by metal contamination and acidification: Crop performance and soil bacterial community responses". In: *Chemosphere* 257, p. 127309. ISSN: 0045-6535. DOI: 10.1016/j.chemosphere.2020.127309.

Lévesque, C. André et al. (2010). "Genome sequence of the necrotrophic plant pathogen Pythium ultimum reveals original pathogenicity mechanisms and effector repertoire". In: *Genome Biology* 11.7. ISSN: 14747596. DOI: 10.1186/gb-2010-11-7-r73.

Ma, Xiaomin et al. (2018). "Spatiotemporal patterns of enzyme activities in the rhizosphere: effects of plant growth and root morphology". In: *Biol. Fertil. Soils* 54.7, pp. 819–828. ISSN: 1432-0789. DOI: 10.1007/s00374-018-1305-6.

Macfadyen, A. (1961). "Improved Funnel-Type Extractors for Soil Arthropods". In: *J. Anim. Ecol.* 30.1, pp. 171–184. ISSN: 0021-8790. URL: http://www.jstor.org/stable/2120.

Madigan, MT et al. (2010). *Brock biology of microorganisms 13th edition*.

Maestre, Fernando T. et al. (2012a). "Plant Species Richness and Ecosystem Multifunctionality in Global Drylands". In: *Science* 335.6065, pp. 214–218. ISSN: 0036-8075. DOI: 10.1126/science.1215442.

Maestre, Fernando T. et al. (2012b). "Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern". In: *J. Ecol.* 100.2, pp. 317–330. ISSN: 0022-0477. DOI: 10.1111/j.1365-2745.2011.01918.x.

Malik, Ashish A. et al. (2017). "Bacterial Physiological Adaptations to Contrasting Edaphic Conditions Identified Using Landscape Scale Metagenomics". In: *mBio* 8.4. ISSN: 2150-7511. DOI: 10.1128/mBio.00799-17.

Malik, Ashish A. et al. (2018). "Land use driven change in soil pH affects microbial carbon cycling processes". In: *Nat. Commun.* 9.3591, pp. 1–10. ISSN: 2041-1723. DOI: 10.1038/s41467-018-05980-1.

Marciano-Cabral, Francine and Guy Cabral (2003). "Acanthamoeba spp. as Agents of Disease in Humans". In: *Clin. Microbiol. Rev.* 16.2, pp. 273–307. ISSN: 0893-8512. DOI: 10.1128/CMR.16.2.273-307.2003.

Marilley, L., U. A. Hartwig, and M. Aragno (1999). "Influence of an Elevated Atmospheric CO2 Content on Soil and Rhizosphere Bacterial Communities Beneath Lolium perenne and Trifolium repens under Field Conditions". In: *Microb. Ecol.* 38.1, pp. 39–49. ISSN: 1432-184X. DOI: 10.1007/s002489900155.

Marotz, Clarisse (2019). "Sequencing Are Unnecessary". In: 67.1, pp. 6–9.

Martiny, Adam C. (2019). "High proportions of bacteria are culturable across major biomes". In: *ISME J.* 13, pp. 2125–2128. ISSN: 1751-7370. DOI: 10.1038/s41396-019-0410-3.

Martiny, Jennifer B. Hughes et al. (2006). "Microbial biogeography: putting microorganisms on the map". In: *Nat. Rev. Microbiol.* 4, pp. 102–112. ISSN: 1740-1534. DOI: 10.1038/nrmicro1341.

Marx, M. C, M Wood, and S. C Jarvis (2001). "A microplate fluorimetric assay for the study of enzyme diversity in soils". In: *Soil Biology and Biochemistry* 33.12, pp. 1633–1640. ISSN: 0038-0717. DOI: 10.1016/S0038-0717(01)00079-7. URL: http://www.sciencedirect.com/science/article/pii/S0038071701000797 (visited on 07/02/2019).

McCarthy, Donal P. et al. (2012). "Financial Costs of Meeting Global Biodiversity Conservation Targets: Current Spending and Unmet Needs". In: *Science* 338.6109, pp. 946–949. ISSN: 0036-8075. DOI: 10.1126/science.1229803.

McGuire, Andrew M., Dennis C. Bryant, and R. Ford Denison (1998). "Wheat Yields, Nitrogen Uptake, and Soil Moisture Following Winter Legume Cover Crop vs. Fallow". In: *Agron. J.* 90.3, pp. 404–410. ISSN: 0002-1962. DOI: 10.2134/agronj1998.00021962009000030015x.

Mee, Michael T. and Harris H. Wang (2012). "Engineering ecosystems and synthetic ecologies". In: *Mol. Biosyst.* 8.10, pp. 2470–2483. ISSN: 1742-2051. DOI: 10.1039/C2MB25133G.

Minasny, Budiman et al. (2017). "Soil carbon 4 per mille". In: *Geoderma* 292, pp. 59–86. ISSN: 0016-7061. DOI: 10.1016/j.geoderma.2017.01.002.

Naeem, Shahid et al. (1994). "Declining biodiversity can alter the performance of ecosystems". In: *Nature* 368.6473, pp. 734–737.

Nannipieri, P et al. (2003). "Microbial diversity and soil functions." In: *European Journal of Soil Science* 54.4, p. 655. ISSN: 13510754. DOI: 10.1046/j.1365-2389.2003.00556.x. URL: 10.1046/j.1351-0754.2003.0556.x%5Cnhttp://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=11414859&site=ehost-live.

Nasseri, AT et al. (2011). "Single cell protein: production and process". In: *American Journal of food technology* 6.2, pp. 103–116.

Nielsen, U. N. et al. (2011). "Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships". In: *Eur. J. Soil Sci.* 62.1, pp. 105–116. ISSN: 1351-0754. DOI: 10.1111/j.1365-2389.2010.01314.x.

Nottingham, Andrew T. et al. (2016). "Temperature sensitivity of soil enzymes along an elevation gradient in the Peruvian Andes". In: *Biogeochemistry* 127.2-3, pp. 217–230. ISSN: 1573515X. DOI: 10.1007/s10533-015-0176-2.

Nunes, J. S. et al. (2012). "Impact of Land Degradation on Soil Microbial Biomass and Activity in Northeast Brazil". en. In: *Pedosphere* 22.1, pp. 88–95. ISSN: 1002-0160. DOI: 10.1016/S1002-0160(11)60194-X. URL: http://www.sciencedirect.com/science/article/pii/S100201601160194X (visited on 11/27/2019).

Nyyssönen, Mari et al. (2013). "Coupled high-throughput functional screening and next generation sequencing for identification of plant polymer decomposing enzymes in metagenomic libraries". In: *Frontiers in Microbiology* 4.SEP, pp. 1–14. ISSN: 1664302X. DOI: 10.3389/fmicb.2013.00282.

Oertel, Cornelius et al. (2016). "Greenhouse gas emissions from soils—A review". In: *Geochemistry* 76.3, pp. 327–352. ISSN: 0009-2819. DOI: 10.1016/j.chemer.2016.04.002.

Oksanen, Roeland Kindt Jari (2008). *vegan - Community Ecology Package*. URL: http://vegan.r-forge.r-project.org/.

Olson, Kenneth, Stephen A Ebelhar, and James M Lang (2014). "Long-term effects of cover crops on crop yields, soil organic carbon stocks and sequestration". In: *Open Journal of Soil Science* 2014.

Orgiazzi, Alberto, Richard D Bardgett, Edmundo Barrios, et al. (2016). *Global soil biodiversity atlas*. European Commission.

Orgiazzi, Alberto et al. (2015). "Soil biodiversity and DNA barcodes: opportunities and challenges". en. In: *Soil Biology and Biochemistry* 80, pp. 244–250. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2014.10.014. URL: http://www.sciencedirect.com/science/article/pii/S0038071714003617 (visited on 11/22/2019).

Paine, R. T. (1995). "A Conversation on Refining the Concept of Keystone Species". In: *Conserv. Biol.* 9.4, pp. 962–964. ISSN: 0888-8892. DOI: 10.1046/j.1523-1739.1995.09040962.x.

Paoletti, Maurizio G. et al. (1991). "Invertebrates as bioindicators of soil use". In: *Agric. Ecosyst. Environ.* 34.1, pp. 341–362. ISSN: 0167-8809. DOI: 10.1016/0167-8809(91)90120-M.

Penton, C. Ryan et al. (2014). "Fungal Community Structure in Disease Suppressive Soils Assessed by 28S LSU Gene Sequencing". en. In: *PLOS ONE* 9.4, e93893. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0093893. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0093893 (visited on 01/07/2020).

Pettersson, Marie and Erland Bååth (2013). "Importance of Inoculum Properties on the Structure and Growth of Bacterial Communities during Recolonisation of Humus Soil with Different pH". In: *Microb. Ecol.* 66.2, pp. 416–426. ISSN: 1432-184X. DOI: 10.1007/s00248-013-0208-1.

Philippot, Laurent et al. (2013). "Going back to the roots: the microbial ecology of the rhizosphere". In: *Nat. Rev. Microbiol.* 11.11, pp. 789–799. ISSN: 1740-1534. DOI: 10.1038/nrmicro3109.

Pierzynski, Gary et al. (2017). "Threats to soils: Global trends and perspectives". In: *Global Land Outlook Working Paper*, p. 28. URL: https://static1.squarespace.

com/static/5694c48bd82d5e9597570999/t/5931752920099eabdb9b6a7a/1496413492935/Threats+to+Soils__Pierzynski_Brajendra.pdf.

Pineda, Ana, Ian Kaplan, and T Martijn Bezemer (2017). "Steering soil microbiomes to suppress aboveground insect pests". In: *Trends in Plant Science* 22.9, pp. 770–778.

Poeplau, Christopher and Axel Don (2015). "Carbon sequestration in agricultural soils via cultivation of cover crops–A meta-analysis". In: *Agriculture, Ecosystems & Environment* 200, pp. 33–41.

Poschlod, Peter and Michiel F WallisDeVries (2002). "The historical and socioeconomic perspective of calcareous grasslands—lessons from the distant and recent past". en. In: *Biological Conservation* 104.3, pp. 361–376. ISSN: 00063207. DOI: 10.1016/S0006-3207(01)00201-4. URL: https://linkinghub.elsevier.com/retrieve/pii/S0006320701002014 (visited on 08/30/2019).

Potthoff, Martin et al. (2006). "Soil microbial community composition as affected by restoration practices in California grassland". en. In: *Soil Biology and Biochemistry* 38.7, pp. 1851–1860. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2005.12.009. URL: http://www.sciencedirect.com/science/article/pii/S0038071706000642 (visited on 11/06/2019).

Powell, Jeff R. and Matthias C. Rillig (2018). "Biodiversity of arbuscular mycorrhizal fungi and ecosystem function". In: *New Phytologist*, pp. 1059–1075. ISSN: 14698137. DOI: 10.1111/nph.15119.

Powlson, D. S. et al. (2012). "The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study". In: *Agric. Ecosyst. Environ.* 146.1, pp. 23–33. ISSN: 0167-8809. DOI: 10.1016/j.agee.2011.10.004.

Powlson, David S. et al. (2014). "Limited potential of no-till agriculture for climate change mitigation". In: *Nat. Clim. Change* 4.8, pp. 678–683. ISSN: 1758-6798. DOI: 10.1038/nclimate2292.

Prechsl, Ulrich E. et al. (2017). "Assessing the environmental impacts of cropping systems and cover crops: Life cycle assessment of FAST, a long-term arable

farming field experiment". en. In: *Agricultural Systems* 157, pp. 39–50. ISSN: 0308-521X. DOI: 10.1016/j.agsy.2017.06.011. URL: http://www.sciencedirect.com/science/article/pii/S0308521X16302748 (visited on 11/06/2019).

Pretty, Jules et al. (2018). "Global assessment of agricultural system redesign for sustainable intensification". In: *Nat. Sustainability* 1.8, pp. 441–446. ISSN: 2398-9629. DOI: 10.1038/s41893-018-0114-0.

Prommer, Judith et al. (2019). "Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity". en. In: *Global Change Biology*. ISSN: 1354-1013, 1365-2486. DOI: 10.1111/gcb.14777. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/gcb.14777 (visited on 09/02/2019).

Puissant, Jérémy et al. (2019). "The pH optimum of soil exoenzymes adapt to long term changes in soil pH". en. In: *Soil Biology and Biochemistry* 138, p. 107601. ISSN: 00380717. DOI: 10.1016/j.soilbio.2019.107601. URL: https://linkinghub.elsevier.com/retrieve/pii/S0038071719302652 (visited on 10/01/2019).

Pulleman, Mirjam et al. (2012). "Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches". In: *Current Opinion in Environmental Sustainability* 4.5, pp. 529–538. ISSN: 1877-3435. DOI: 10.1016/j.cosust.2012.10.009.

Putte, An Van den et al. (2010). "Assessing the effect of soil tillage on crop growth: A meta-regression analysis on European crop yields under conservation agriculture". In: *Eur. J. Agron.* 33.3, pp. 231–241. ISSN: 1161-0301. DOI: 10.1016/j.eja.2010.05.008.

Pywell, Richard F. et al. (2007). "Enhancing diversity of species-poor grasslands: An experimental assessment of multiple constraints". In: *Journal of Applied Ecology* 44.1, pp. 81–94. ISSN: 00218901. DOI: 10.1111/j.1365-2664.2006.01260.x.

Quince, Christopher et al. (2017). "Shotgun metagenomics, from sampling to analysis". In: *Nat. Biotechnol.* 35, pp. 833–844. ISSN: 1546-1696. DOI: 10.1038/nbt.3935.

Quinton, John N and JA Catt (2004). "The effects of minimal tillage and contour cultivation on surface runoff, soil loss and crop yield in the long-term Woburn

Erosion Reference Experiment on sandy soil at Woburn, England". In: *Soil Use and Management* 20.3, pp. 343–349.

R Core Team, R Foundation for Statistical Computing (2017). *R: A Language and Environment for Statistical Computing*. Vienna, Austria. URL: https://www.r-project.org/.

Raich, James W and William H Schlesinger (1992). "The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate". In: *Tellus B* 44.2, pp. 81–99.

Ramirez, Kelly S., Joseph M. Craine, and Noah Fierer (2012). "Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes". In: *Global Change Biology* 18.6, pp. 1918–1927. ISSN: 13541013. DOI: 10.1111/j.1365-2486.2012.02639.x.

Ramirez, Kelly S et al. (2014). "Biogeographic patterns in below-ground diversity in New York City ' s Central Park are similar to those observed globally Biogeographic patterns in below-ground diversity in New York City ' s Central Park are similar to those observed globally". In: *Proceedings of the Royal Society B* 281, p. 20141988. ISSN: 0962-8452. DOI: http://dx.doi.org/10.1098/rspb.2014.1988.

Ramirez, Kelly S. et al. (2017). "Detecting macroecological patterns in bacterial communities across independent studies of global soils". In: *Nat. Microbiol.* 3.2, pp. 189–196. ISSN: 2058-5276. DOI: 10.1038/s41564-017-0062-x.

Raskin, Ilya, Burt D Ensley, et al. (2000). *Phytoremediation of toxic metals.* John Wiley and Sons.

Redhead, John W. et al. (2014). "The natural regeneration of calcareous grassland at a landscape scale: 150 years of plant community re-assembly on Salisbury Plain, UK". en. In: *Applied Vegetation Science* 17.3, pp. 408–418. ISSN: 1654-109X. DOI: 10.1111/avsc.12076. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/avsc.12076 (visited on 07/12/2019).

Reed, ST and DC Martens (1996). "Methods of Soil Analysis Part 3—Chemical Methods". In: *Methods of Soil Analysis Part 3—Chemical Methods*, pp. i–xxi. ISSN: 2163-2804. DOI: 10.2136/sssabookser5.3.frontmatter. URL: https://dl.sciencesocieties.

org/publications/books/articles/sssabookseries/methodsofsoilan3/frontmatter.

Reid, Walter V. and Et Al. (2005). *Millennium Ecosystem Assessment*. ISBN: 978-1-59726-040. URL: https://www.wri.org/publication/millennium-ecosystem-assessment-0.

Rembiałkowska, Ewa (2007). "Quality of plant products from organic agriculture". In: *Journal of the Science of Food and Agriculture* 87.15, pp. 2757–2762.

Ridding, Lucy E., John W. Redhead, and Richard F. Pywell (2015). "Fate of semi-natural grassland in England between 1960 and 2013: A test of national conservation policy". In: *Global Ecology and Conservation* 4, pp. 516–525. ISSN: 2351-9894. DOI: 10.1016/j.gecco.2015.10.004. URL: http://www.sciencedirect.com/science/article/pii/S2351989415300184 (visited on 07/15/2019).

Rillig, Matthias C. and Anika Lehmann (2019). "Exploring the agricultural parameter space for crop yield and sustainability". In: *New Phytologist*, pp. 517–519. DOI: 10.1111/nph.15744.

Rillig, Matthias C. et al. (2015). "Interchange of entire communities: microbial community coalescence". In: *Trends Ecol. Evol.* 30.8, pp. 470–476. ISSN: 0169-5347. DOI: 10.1016/j.tree.2015.06.004.

Ritz, Karl et al. (2009). "Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development". In: *Ecol. Indic.* 9.6, pp. 1212–1221. ISSN: 1470-160X. DOI: 10.1016/j.ecolind.2009.02.009.

Roberts, David (2019). *Ordination and Multivariate Analysis for Ecology*. URL: http://ecology.msu.montana.edu/labdsv/R (visited on 08/04/2019).

Rockström, Johan et al. (2009). "A safe operating space for humanity". In: *Nature* 461.7263, pp. 472–475. ISSN: 1476-4687. DOI: 10.1038/461472a.

Rosenberg, Eugene et al. (2014). "The prokaryotes: Actinobacteria". In: *The Prokaryotes: Actinobacteria*, pp. 1–1061. DOI: 10.1007/978-3-642-30138-4.

Rossi, V. et al. (2005). "Growth and sporulation of Stemphylium vesicarium, the causal agent of brown spot of pear, on herb plants of orchard lawns". In: *Eur. J. Plant Pathol.* 111.4, pp. 361–370. ISSN: 1573-8469. DOI: 10.1007/s10658-004-5273-3.

Rousk, Johannes et al. (2010). "Soil bacterial and fungal communities across a pH gradient in an arable soil". In: *ISME J.* 4.10, pp. 1340–1351. ISSN: 1751-7370. DOI: 10.1038/ismej.2010.58.

Rowley, Mike C., Stéphanie Grand, and Éric P. Verrecchia (2017). "Calcium-mediated stabilisation of soil organic carbon". In: *Biogeochemistry*, pp. 1–23. ISSN: 1573515X. DOI: 10.1007/s10533-017-0410-1.

Russell, Jonathan R. et al. (2011). "Biodegradation of Polyester Polyurethane by Endophytic Fungi". en. In: *Applied and Environmental Microbiology* 77.17, pp. 6076–6084. ISSN: 0099-2240, 1098-5336. DOI: 10.1128/AEM.00521-11. URL: https://aem.asm.org/content/77/17/6076 (visited on 07/17/2019).

Saccá, Maria Ludovica et al. (2017). *Ecosystem Services Provided By Soil Microorganisms*. Springer, Cham. ISBN: 978-3-319-63335-0. DOI: 10.1007/978-3-319-63336-7_2.

Sala, Osvaldo E. et al. (2000). "Global Biodiversity Scenarios for the Year 2100". In: *Science* 287.5459, pp. 1770–1774. ISSN: 0036-8075. DOI: 10.1126/science.287.5459.1770.

Saviozzi, Alessandro et al. (2001). "A comparison of soil quality in adjacent cultivated, forest and native grassland soils". In: *Plant and soil* 233.2, pp. 251–259.

Schnittler, Martin et al. (2012). "Myxomycetes and myxomycete-like organisms". In: *Englers Syllabus of Plant Families* 4, pp. 40–88. URL: https://www.researchgate.net/publication/302506360_Myxomycetes_and_myxomycete-like_organisms.

Schröter, Matthias et al. (2017). "Citizen science for assessing ecosystem services: Status, challenges and opportunities". en. In: *Ecosystem Services* 28, pp. 80–94. ISSN: 2212-0416. DOI: 10.1016/j.ecoser.2017.09.017. URL: http://www.sciencedirect.com/science/article/pii/S2212041617302462 (visited on 02/06/2020).

Seaton, Fiona M. et al. (2019). "Plant and soil communities are associated with the response of soil water repellency to environmental stress". In: *Science of The Total Environment* 687, pp. 929–938. ISSN: 00489697. DOI: 10.1016/j.scitotenv.2019.06.052. URL: https://linkinghub.elsevier.com/retrieve/pii/S0048969719326130.

Shafiee, Rasool, Iraj Nahvi, and Giti Emtiazi (2005). "Bioconversion of raw starch to SCP by coculture of Cryptococcus aerius and Saccharomyces cerevisiae". In: *Journal of Biological Sciences* 5.6, pp. 717–723.

Shendure, Jay et al. (2017). "DNA sequencing at 40: past, present and future". In: *Nature* 550, pp. 345–353. ISSN: 1476-4687. DOI: 10.1038/nature24286.

Sigler, Lynne, Trevor C Lumley, and Randolph S Currah (2000). *New species and records of saprophytic ascomycetes (Myxotrichaceae) from decaying logs in the boreal forest*. Tech. rep., pp. 495–502. URL: https://www.uamh.ca/Research/_/media/uamh/Research/Publications/UamhPubs/2000_Sigler_etal_Myxotrichaceae_in_decaying_logs_Mycoscience.pdf.

Silvertown, Jonathan (2009). "A new dawn for citizen science". en. In: *Trends in Ecology & Evolution* 24.9, pp. 467–471. ISSN: 0169-5347. DOI: 10.1016/j.tree.2009.03.017. URL: http://www.sciencedirect.com/science/article/pii/S016953470900175X (visited on 02/06/2020).

Sinsabaugh, Robert L. et al. (2008). "Stoichiometry of soil enzyme activity at global scale". In: *Ecol. Lett.* 11.11, pp. 1252–1264. ISSN: 1461-023X. DOI: 10.1111/j.1461-0248.2008.01245.x.

Smith, P. et al. (2015). "Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils". In: *Soil* 1.2, pp. 665–685. ISSN: 2199398X. DOI: 10.5194/soil-1-665-2015.

Smith, Pete et al. (2016). "Global change pressures on soils from land use and management". In: *Global Change Biology* 22.3, pp. 1008–1028. ISSN: 13652486. DOI: 10.1111/gcb.13068.

Spohn, Marie (2016). "Element cycling as driven by stoichiometric homeostasis of soil microorganisms". In: *Basic Appl. Ecol.* 17.6, pp. 471–478. ISSN: 1439-1791. DOI: 10.1016/j.baae.2016.05.003.

Šťursová, Martina and Petr Baldrian (2011). "Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity". In: *Plant Soil* 338.1, pp. 99–110. ISSN: 1573-5036. DOI: 10.1007/s11104-010-0296-3.

Stark, Sari, Minna K. Männistö, and Anu Eskelinen (2014). "Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor

tundra soils". In: *Plant Soil* 383.1, pp. 373–385. ISSN: 1573-5036. DOI: 10.1007/s11104-014-2181-y.

Steduto, P. et al. (2002). "Automated closed-system canopy-chamber for continuous field-crop monitoring of CO2and H2O fluxes". In: *Agricultural and Forest Meteorology* 111.3, pp. 171–186. ISSN: 01681923. DOI: 10.1016/S0168-1923(02)00023-0.

Stone, D. et al. (2016). "A method of establishing a transect for biodiversity and ecosystem function monitoring across Europe". In: *Applied Soil Ecology* 97, pp. 3–11. ISSN: 09291393. DOI: 10.1016/j.apsoil.2015.06.017.

Sutton, Mark A. et al. (2011). "Too much of a good thing". In: *Nature* 472, pp. 159–161. ISSN: 1476-4687. DOI: 10.1038/472159a.

Tedersoo, Leho et al. (2014). "Global diversity and geography of soil fungi". In: *Science* 346.6213, p. 1256688. ISSN: 0036-8075. DOI: 10.1126/science.1256688.

Thies, Janice E. (2015). "Molecular Approaches to Studying the Soil Biota". In: *Soil Microbiology, Ecology and Biochemistry*. Elsevier, pp. 151–185. ISBN: 978-0-12-415955-6. DOI: 10.1016/B978-0-12-415955-6.00006-2. URL: https://linkinghub.elsevier.com/retrieve/pii/B9780124159556000062 (visited on 08/13/2020).

Thompson, Luke R. et al. (2017). "A communal catalogue reveals Earth's multiscale microbial diversity". en. In: *Nature* 551.7681, pp. 457–463. ISSN: 1476-4687. DOI: 10.1038/nature24621. URL: https://www.nature.com/articles/nature24621 (visited on 07/28/2019).

Thomson, Bruce C. et al. (2010). "Vegetation affects the relative abundances of dominant soil bacterial taxa and soil respiration rates in an upland grassland soil". In: *Microbial Ecology* 59.2, pp. 335–343. ISSN: 00953628. DOI: 10.1007/s00248-009-9575-z.

Thomson, Bruce C. et al. (2015). "Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites". en. In: *Soil Biology and Biochemistry* 88, pp. 403–413. ISSN: 0038-0717. DOI: 10.1016/j.soilbio.2015.06.012. URL: http://www.sciencedirect.com/science/article/pii/S0038071715002175 (visited on 11/21/2019).

Thornton, R. H. (1965). "Studies of fungi in pasture soils". In: *New Zealand Journal of Agricultural Research* 8.3, pp. 417–449. ISSN: 0028-8233. DOI: 10.1080/00288233.

1965.10419888. URL: https://doi.org/10.1080/00288233.1965.10419888 (visited on 11/29/2019).

Tilman, D. et al. (2011). "Global food demand and the sustainable intensification of agriculture". en. In: *Proceedings of the National Academy of Sciences* 108.50, pp. 20260–20264. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/pnas.1116437108. URL: http://www.pnas.org/cgi/doi/10.1073/pnas.1116437108 (visited on 01/14/2020).

Tilman, David et al. (1997). "The Influence of Functional Diversity and Composition on Ecosystem Processes". In: *Science* 277.5330, pp. 1300–1302. ISSN: 0036-8075. DOI: 10.1126/science.277.5330.1300.

Tilman, David et al. (2002). "Agricultural sustainability and intensive production practices". In: *Nature* 418.6898, pp. 671–677. ISSN: 1476-4687. DOI: 10.1038/nature01014.

Toju, Hirokazu et al. (2018). "Core microbiomes for sustainable agroecosystems". In: *Nat. Plants* 4.5, pp. 247–257. ISSN: 2055-0278. DOI: 10.1038/s41477-018-0139-4.

Toth, J, EJ Nurthen, and KY Chan (1988). "A simple wick method for watering potted plants which maintains a chosen moisture regime". In: *Australian Journal of Experimental Agriculture* 28.6, pp. 805–808.

Trinci, Anthony P. J. (1992). "Myco-protein: A twenty-year overnight success story". In: *Mycol. Res.* 96.1, pp. 1–13. ISSN: 0953-7562. DOI: 10.1016/S0953-7562(09)80989-1.

Trumbore, Susan (2000). "AGE OF SOIL ORGANIC MATTER AND SOIL RESPIRATION: RADIOCARBON CONSTRAINTS ON BELOWGROUND C DYNAMICS". In: *Ecol. Appl.* 10.2, pp. 399–411. ISSN: 1051-0761. DOI: 10.1890/1051-0761(2000)010[0399:AOSOMA]2.0.CO;2.

Tscherko, D and E Kandeler (1999). "Classification and monitoring ofsoll microbial biomass, N-mineralization and enzyme activities to indicate environmental changes". In: *Die Bodenkultur* 215.50, p. 4.

Tsiafouli, Maria A. et al. (2015). "Intensive agriculture reduces soil biodiversity across Europe". en. In: *Global Change Biology* 21.2, pp. 973–985. ISSN: 1365-2486. DOI: 10.1111/gcb.12752. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/gcb.12752 (visited on 01/07/2020).

Turley, D. B. et al. (2003). "Long-term straw management effects on yields of sequential wheat (Triticum aestivum L.) crops in clay and silty clay loam soils in England". In: *Soil Tillage Res.* 71.1, pp. 59–69. ISSN: 0167-1987. DOI: 10.1016/S0167-1987(03)00018-7.

Turner, Benjamin L. (2010). "Variation in pH Optima of Hydrolytic Enzyme Activities in Tropical Rain Forest Soils". en. In: *Applied and Environmental Microbiology* 76.19, pp. 6485–6493. ISSN: 0099-2240, 1098-5336. DOI: 10.1128/AEM.00560-10. URL: https://aem.asm.org/content/76/19/6485 (visited on 10/28/2019).

Twomlow, Steve et al. (2010). "Micro-dosing as a pathway to Africa's Green Revolution: evidence from broad-scale on-farm trials". en. In: *Nutrient Cycling in Agroecosystems* 88.1, pp. 3–15. ISSN: 1573-0867. DOI: 10.1007/s10705-008-9200-4. URL: https://doi.org/10.1007/s10705-008-9200-4 (visited on 01/14/2020).

Urbanski, Livia et al. (2018). "Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales". In: *Geoderma* 333.July 2018, pp. 149–162. ISSN: 00167061. DOI: 10.1016/j.geoderma.2018.07.026. URL: https://doi.org/10.1016/j.geoderma.2018.07.026.

Urich, Tim et al. (2008). "Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome". In: *PLoS One* 3.6, e2527. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0002527.

Vincent-Caboud, Laura et al. (2017). "Overview of Organic Cover Crop-Based No-Tillage Technique in Europe: Farmers' Practices and Research Challenges". In: *Agriculture* 7.5, p. 42. ISSN: 2077-0472. DOI: 10.3390/agriculture7050042. URL: http://www.mdpi.com/2077-0472/7/5/42.

Vogel, Timothy M. et al. (2009). "TerraGenome: a consortium for the sequencing of a soil metagenome". en. In: *Nature Reviews Microbiology* 7.4, pp. 252–252. ISSN: 1740-1534. DOI: 10.1038/nrmicro2119. URL: https://www.nature.com/articles/nrmicro2119 (visited on 11/27/2019).

Vries, Franciska T. de et al. (2012). "Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities". In: *Ecol. Lett.* 15.11, pp. 1230–1239. ISSN: 1461-023X. DOI: 10.1111/j.1461-0248.2012.01844.x.

Wagner, Markus et al. (2019). "Species indicators for naturally-regenerating and old calcareous grassland in southern England". In: *Ecological Indicators* 101.January,

pp. 804–812. ISSN: 1470160X. DOI: 10.1016/J.ECOLIND.2019.01.082. URL: https://www.sciencedirect.com/science/article/pii/S1470160X19301001?dgcid=author.

Wallace, Henry A (1910). "Relation between live stock farming and the fertility of the land". In:

Wallenstein, Matthew D. and Michael N. Weintraub (2008). "Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes". In: *Soil Biology and Biochemistry*. ISSN: 00380717. DOI: 10.1016/j.soilbio.2008.01.024.

Walsh, Corinne M. et al. (2019). "A Global Survey of Mycobacterial Diversity in Soil". In: *Appl. Environ. Microbiol.* 85.17. ISSN: 0099-2240. DOI: 10.1128/AEM.01180-19.

Walters, William et al. (2016). "Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys". en. In: *mSystems* 1.1, e00009–15. ISSN: 2379-5077. DOI: 10.1128/mSystems.00009-15. URL: https://msystems.asm.org/content/1/1/e00009-15 (visited on 07/26/2019).

Wan, Wenjie et al. (2020). "Responses of the Rhizosphere Bacterial Community in Acidic Crop Soil to pH: Changes in Diversity, Composition, Interaction, and Function". In: *Sci. Total Environ.* 700, p. 134418. ISSN: 1879-1026. DOI: 10.1016/j.scitotenv.2019.134418.

Watkinson, Sarah C. (2016). "Mutualistic Symbiosis Between Fungi and Autotrophs". In: *The Fungi: Third Edition*. Academic Press, pp. 205–243. ISBN: 978-0-12-382034-1. DOI: 10.1016/B978-0-12-382034-1.00007-4. URL: https://www.sciencedirect.com/science/article/pii/B9780123820341000074.

Wayman, Sandra et al. (2014). "The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems". In: *Renew Agr Food Syst* 30, pp. 450–460.

Weintraub, Michael N. et al. (2007). "The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem". In: *Oecologia* 154.2, pp. 327–338. ISSN: 1432-1939. DOI: 10.1007/s00442-007-0804-1. URL: https://doi.org/10.1007/s00442-007-0804-1.

Weisser, Wolfgang W. et al. (2017). "Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions". In: *Basic Appl. Ecol.* 23, pp. 1–73. ISSN: 1439-1791. DOI: 10.1016/j.baae.2017.06.002.

Wenzel, Walter W. (2009). *Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils*. Vol. 321. 1. Dordrecht, The Netherlands: Springer Netherlands. DOI: 10.1007/s11104-008-9686-1.

Wertz, Sophie et al. (2006). "Maintenance of soil functioning following erosion of microbial diversity". In: *Environmental Microbiology* 8.12, pp. 2162–2169. ISSN: 14622912. DOI: 10.1111/j.1462-2920.2006.01098.x.

Westhoek, Henk et al. (2014). "Food choices, health and environment: Effects of cutting Europe's meat and dairy intake". In: *Global Environ. Change* 26, pp. 196–205. ISSN: 0959-3780. DOI: 10.1016/j.gloenvcha.2014.02.004.

White, DC et al. (1979). "Determination of the sedimentary microbial biomass by extractible lipid phosphate". In: *Oecologia* 40.1, pp. 51–62.

Williams, Stacey M. and Ray R. Weil (2004). "Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop". In: *Soil Sci. Soc. Am. J.* 68.4, pp. 1403–1409. ISSN: 0361-5995. DOI: 10.2136/sssaj2004.1403.

Wohl, Debra L., Satyam Arora, and Jessica R. Gladstone (2004). "FUNCTIONAL REDUNDANCY SUPPORTS BIODIVERSITY AND ECOSYSTEM FUNCTION IN A CLOSED AND CONSTANT ENVIRONMENT". In: *Ecology* 85.6, pp. 1534–1540. ISSN: 0012-9658. DOI: 10.1890/03-3050.

Wollum, A. G. (1994). "Soil Sampling for Microbiological Analysis". In: *Methods of Soil Analysis*. Chichester, England, UK: John Wiley & Sons, Ltd, pp. 1–14. ISBN: 978-0-89118865-0. DOI: 10.2136/sssabookser5.2.c1.

Wubs, E R Jasper et al. (2016). "Soil inoculation steers restoration of terrestrial ecosystems". In: *Nature Plants* 2.July, p. 16107. ISSN: 2055-0278. DOI: 10.1038/nplants.2016.107. URL: http://www.nature.com/articles/nplants2016107.

Xue, Allen G. (2007). "Biological Control of Pathogens Causing Root Rot Complex in Field Pea Using Clonostachys rosea Strain ACM941". In: *APS Publications*. URL: https://apsjournals.apsnet.org/doi/abs/10.1094/PHYTO.2003.93.3.329.

Yin, Rui et al. (2020). "Climate change and intensive land use reduce soil animal biomass via dissimilar pathways". In: *eLife*. DOI: 10.7554/eLife.54749.

Zavaleta, Erika S et al. (2010). "Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity". In: *Proceedings of the National Academy of Sciences* 107.4, pp. 1443–1446.

Zechmeister-Boltenstern, Sophie et al. (2015). "The application of ecological stoichiometry to plant–microbial–soil organic matter transformations". In: *Ecol. Monogr.* 85.2, pp. 133–155. ISSN: 0012-9615. DOI: 10.1890/14-0777.1.

Zhalnina, Kateryna et al. (2013). "Ca. Nitrososphaera and Bradyrhizobium are inversely correlated and related to agricultural practices in long-term field experiments". English. In: *Frontiers in Microbiology* 4. ISSN: 1664-302X. DOI: 10.3389/fmicb.2013.00104. URL: https://www.frontiersin.org/articles/10.3389/fmicb.2013.00104/full (visited on 07/19/2019).

Zhalnina, Kateryna et al. (2014). "Soil pH Determines Microbial Diversity and Composition in the Park Grass Experiment". In: *Microbial Ecology* 69.2, pp. 395–406. ISSN: 00953628. DOI: 10.1007/s00248-014-0530-2.

Zhang, Zhaojing et al. (2017). "Soil bacterial quantification approaches coupling with relative abundances reflecting the changes of taxa". In: *Scientific Reports* 7.1, pp. 1–11. ISSN: 20452322. DOI: 10.1038/s41598-017-05260-w.

Zhao, Cancan et al. (2016). "Soil microbial community composition and respiration along an experimental precipitation gradient in a semiarid steppe." In: *Scientific reports* 6.March, p. 24317. ISSN: 2045-2322. DOI: 10.1038/srep24317. URL: http://www.ncbi.nlm.nih.gov/pubmed/27074973%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4830937.