

AGROECOSYSTEM DIVERSIFICATION FOR SUSTAINABILITY: THE EFFECTS OF CROP ROTATION ON SOIL MICROBIAL DIVERSITY, FERTILITY AND YIELD

by
Zander Samuel Venter

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Supervisor: Dr. Heidi-Jayne Hawkins
Co-supervisor: Prof. Karin Jabobs
Faculty of AgriSciences

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ABSTRACT

Agricultural intensification can involve the simplification of agroecosystems to crop monocultures requiring agrochemicals to maintain soil fertility and control pests. This can have negative impacts on the ecosystem services flowing to and from agroecosystems and thus, more sustainable management practices are necessary. Broader ecological theories propose that ecosystem biodiversity is important for ecosystem function. Within agriculture, increasing aboveground diversity through crop rotation, a component of conservation agriculture, can enhance the ecosystem services supporting an increase in cash-crop yield. The extent to which the belowground microbial diversity can be manipulated through crop rotations and may facilitate the yield increase is unclear. This thesis aimed at investigating (1) the relationship between above and belowground diversity in crop rotation systems, and (2) the relationship between diversity in (1) and agroecosystem function in terms of soil fertility, including nitrogen fertility, and crop yield.

A quantitative review of the literature using a meta-analysis of 27 studies from around the world found that soils under a higher diversity of crops in rotation produced higher microbial richness (+15.11%) and diversity (+3.36%) scores. This effect was significantly influenced by the type of microbial analysis method used, the length of the study trial, and the percentage annual ground cover. There was a high between-study heterogeneity and no correlation to soil nitrogen fertility.

A field study at Langgewens Experimental Farm (Western Cape Department of Agriculture's 19-year wheat-legume crop rotation trial in the Swartland Local Municipality of South Africa) added to the meta-analysis. Community level physiological profiling (CLPP) and automated rRNA intergenic spacer analysis (ARISA) were used as measures of functional and genetic microbial diversity, respectively. Increasing crop diversity through rotations of wheat with medic (Wm) or a combination of medic and clover (Wmc) resulted in greater wheat plant stem length and N concentrations when compared to wheat monoculture (WW). This effect seemed to be less linked with microbial diversity per se than with the *Rhizobium* species present because both microbial analyses found no differences in soil microbial activity, richness or diversity with increasing crop diversity. The lack of relationship between above and belowground diversity is likely due to other abiotic drivers of microbial community structure such as P availability, Na and K excess, and pH, all of which correlated to microbial activity and functional richness in our study. The role that microbial diversity plays in the

agroecosystem diversity-function relationship remains complex as revealed by the lack of correlation between functional and genetic diversity scores. However, the relation between crop diversity and functional components including wheat yield and soil N followed a hump-shaped curve.

The results of this thesis suggest that soil biodiversity and function are decoupled in agroecosystems. This provides support for the hypothesis that ecosystem function may be a product of either specific productive species (selection effect), or the facilitative interaction of multiple species (complementarity effect). Further investigation into the role of specific functional microbial groups in the yield increase of crop rotation systems using next-generation sequencing is required.

OPSOMMING

Die verhoging van landbou intensiteit behels die omskakeling van landbou ekosisteme na monokultuur, wat landbou-chemikalieë vereis om grondvrugbaarheid te handhaag en peste in toom te hou. Hierdie praktyke het 'n negatiewe impak op ekosisteedienste wat vloeï van of na die landbou-ekosisteme. Dit vereis meer volhoubare landboubestuurpraktyke. Wier ekologiese teorieë stel voor dat ekosisteediversiteit 'n belangrike rol speel in die funksie van die ekosisteedienste. Binne landboupraktyke, kan 'n toename in bogrondse diversiteit deur wisselbou, die ekosisteedienste verbeter, wat lei tot 'n toename in die opbrengs in kontant gewasse. Die mate waartoe die ondergrondse mikrobiële diversiteit gemanipuleer kan word, is nog onduidelik. Hierdie tesis beoog om (1) die verwantskap tussen die bogrondse en ondergrondse diversiteit in wisselbousisteme, en (2) die verwantskap tussen die diversiteit in (1) en die landbouekosisteedienste funksie te ondersoek in terme van grondvrugbaarheid, insluitend stikstofvrugbaarheid, en gewasopbrengs.

'n Kwantitatiewe oorsig van literatuur deur gebruik te maak van 'n meta-analise van 27 studies van regoor die wêreld, het gevind dat grond met 'n hoër diversiteit van gewasse in wisselbou gelei het tot 'n hoër mikrobiële (+15.11%) tellings, terwyl die effek van diversiteit (+3.36%) nie beduidend was nie. Die effek van diversiteit was beduidend beïnvloed deur die tipe mikrobiële-analitiese metode wat gebruik was, die lengte van die toetsperiode en die persentasie van jaarlikse grondbedekking. Die heterogeniteit tussen studies was hoog en daar was geen korrelasie met grond stikstofvrugbaarheid nie.

'n Veldstudie by Langgewens proefplaas (Weskaap Departement van Landbou se 19-jaar koring-peulplant wisselbou toets in die Swartland plaaslike munisipaliteit, Suid-Afrika) is bygevoeg tot die meta-analiese. Gemeenskapvlak fisiologiese profiel bepaling (GVFP) en automatiese rRNS intergeniese spasie analiese (ARISA) is onderskeidelik gebruik as maatstawwe van funksionele en genetiese mikrobiële diversiteit. Toenemende gewas diversiteit deur wisselbou van koring met medic (WM) of 'n kombinasie van medic en klawer (Wmc) het 'n groter koringplant stamlengte en N konsentrasie tot gevolg gehad wanneer dit vergelyk word met koring monokultuur (WW). Die effek blyk minder gekoppel te wees met mikrobiële diversiteit per se as met die *Rhizobium* spesies wat betrokke is. Mikrobiële analises van beide sisteme het gevind dat daar geen veranderinge in die grond se mikrobiële aktiwiteit, rykdom of diversiteit is, met toename in gewasdiversiteit nie. Die afwesigheid van 'n verhouding tussen die bogrondse en ondergrondse diversiteit is waarskynlik ook as gevolg van ander abiotiese drywers van die mikrobiële gemeenskapstruktuur soos beskikbaarheid, oormaat in Na en K, en pH, wat alles gekoppel is aan mikrobiële-aktiwiteit en funksionele rykdom in die studie. Die rol wat mikrobiële diversiteit speel in die agro-ekosisteediversiteit

funksie bly kompleks soos uitgewys deur die gebrek aan verwantskap tussen die funksionele en genetiese diversiteitstellings. Nietemin, die verhouding tussen gewas-diversiteit en funksionele komponente, insluitend koringopbrengs en grond N, het 'n bultvormige kurwe gevolg.

Die resultate van hierdie tesis dui daarop dat biodiversiteit en funksie ontkoppel is in landbou-ekosisteme. Dit ondersteun die hipotese dat ekosisteem-funksie 'n produk kan wees van of spesifieke produktiewe spesies (seleksie effek), of die fasiliterende interaksie van verskeie spesies (komplimentêre effek). 'n Verdere ondersoek in die rol van spesifiek funksionele groepe in die opbrengs toename van wisselbou sisteme word benodig deur gebruik te maak van volgende-generasie DNA volgorde bepaling.

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CHAPTER 1: GENERAL INTRODUCTION AND RESEARCH AIMS

The intensification of conventional agricultural practices has been associated with the simplification of cropping systems to monocultures, resulting in a loss of 75% of global agricultural crops over the past 60 years (Kotze and Rose, 2015). Instead of incorporating the natural ecosystem services resulting from diversifying cropping systems, conventional agriculture relies on the use of petrochemicals (pesticides, herbicides and fertilizers) to enhance soil fertility and control pests (Karlen et al., 1994). The practices associated with monocultures pose a threat to the ecosystem services flowing to and from agroecosystems by contributing to soil erosion, pollution of groundwater, release of green-house gases and biodiversity loss (Tilman, 2001). Thus, the simplification of agroecosystems may compromise their sustainability.

In response to the negative effects of the agricultural green revolution, there has emerged a paradigm shift toward sustainability. The adoption of conservation agriculture in crop systems is an example of this shift, and it involves the practice of crop rotation, along with no-till and soil cover, as a means of agroecosystem diversification (Hobbs et al., 2008). Historically, the adoption of crop rotations was largely motivated by the associated yield increase in the cash crop (Bullock et al., 1992). Numerous scientific studies have shown the correlation between yield increase and increasing crop diversity (Smith et al., 2008). The causal mechanisms through which this yield increase is achieved, generally considered components of agroecosystem function, include increased soil fertility (particularly when leguminous plants are used in rotation), maintenance of soil structure, disruption of pest cycles and weed suppression.

It is widely understood that the delivery of these services is mediated by the microorganisms within the soil as they are responsible for many biogeochemical reactions concerning nutrient cycling and climate mitigation (de Vries et al., 2013). Soil microorganisms enhance soil fertility through the mineralisation of limiting nutrients such as nitrogen (N) and phosphorus (P) (Dias et al., 2015). For example, N₂-fixing bacteria and mycorrhizal fungi are responsible for 5 to 20% N and 75% of P acquired in grassland and savannah plants annually (van der Heijden et al., 2008) as well as in the crop plants wheat and carrot (Hawkins et al., 2000). Yet, the extent to which the biodiversity of soil microorganisms, in terms of species richness and relative abundance, affects nutrient cycling and the other ecosystem services associated with crop rotations is unclear.

Within the broader context of diversity-ecosystem function theory, there are a number of hypotheses around the relationship between diversity and function (Figure 1.1). An increase

in crop and/or microbial diversity may result in a linear, curvilinear or poorly curvilinear relationship with the agroecosystem function (Smith et al., 2008; Vitousek and Hooper, 1994). Alternatively, it has been proposed that the well-known hump-shaped relation between biodiversity and function in higher plants may as well be true for soil microbial biodiversity (Anderson, 2003; Nannipieri et al., 2003).

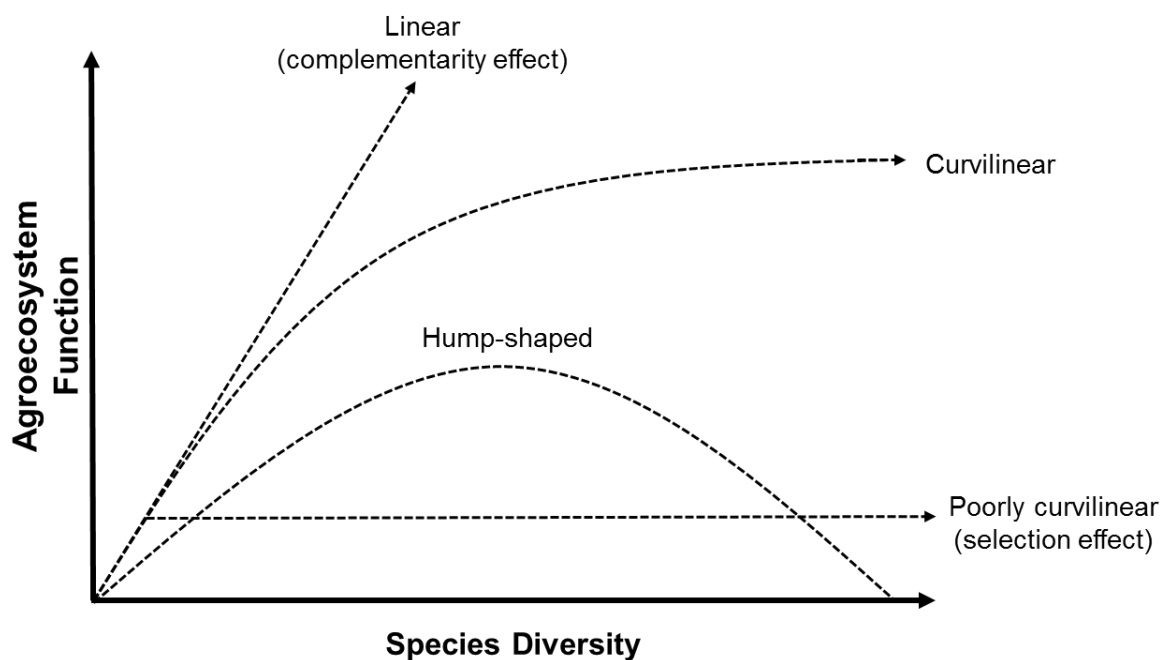


Figure 1.1: Graphical depiction adapted from broader diversity-ecosystem function theories (Anderson, 2003; Nannipieri et al., 2003; Smith et al., 2008; Vitousek and Hooper, 1994) and their possible mechanisms, indicated in brackets (Hooper et al., 2000; Hooper et al., 2005).

Studying the relationship between diversity and function requires the understanding of the mechanisms involved. Broader ecological theories suggest the relationship may be governed by alternative mechanisms, namely the selection effect or complementarity effect (Figure 1.1; Hooper et al., 2000; Hooper et al., 2005). Ecosystem function may be a product of a singular key-stone species such as *N₂-fixing Rhizobia* (the selection effect), or due to the facilitation and niche differentiation associated with a number of species. Another theory related to the complementarity effect hypothesis is that of functional redundancy (Giller et al., 1997). A diversity of species within an ecosystem contributes to its resilience to stress or disturbance due to multiple taxonomic sub-units that can perform the same function and have variable tolerances to stress. For example, experiments have shown that higher microbial diversity results in shorter recovery time in pasture soil communities after short-term and long-term stress (heat and fumigation), i.e. increased resilience (Griffiths et al., 2000). Within

agroecosystems, the aforementioned theoretical mechanisms may be at work in combination, thus providing support for both the decoupling and coupling of biodiversity with function.

Supposing there is a given relationship between biodiversity (aboveground and belowground) and agroecosystem function, it becomes important to understand the drivers of biodiversity if we want to enhance agroecosystem function. Aboveground diversity in agroecosystems can generally be manipulated through increasing or decreasing the number of crops in rotation. Belowground diversity (microbial diversity) is, however, not as easily manipulated through agricultural management practices as there are abiotic drivers (edaphic variables such as soil pH and moisture) which are considered primary drivers (Berg and Smalla, 2009). Yet, plant diversity is also now recognised as an important driver of soil microbial diversity and it has been posited that increasing aboveground diversity through crop rotations can result in corresponding increases in diversity belowground (Hooper et al., 2000). However, there is conflicting evidence in the literature on this relationship. For example, studies using molecular-based methods measuring genetic microbial diversity have shown positive (Yao et al., 2006; Guong et al., 2012), negative (Mathimaran et al., 2007; van Elsas et al., 2002; Yin et al., 2010) and contrasting effects (Alvey et al., 2003; Azziz et al., 2012) of crop rotations on functional microbial diversity.

To date, there is no comprehensive review of the literature examining the link between crop diversity, soil microbial diversity and agroecosystem function. In addition, no studies have examined this dynamic within South Africa. For these reasons, the present thesis aims to investigate the following and is summarised in Figure 1.2:

1. The relationship between above and belowground agroecosystem diversity in terms of crop rotation and microbial diversity respectively.
2. The relationship between agroecosystem diversity and agroecosystem function in terms of soil fertility and crop yield.

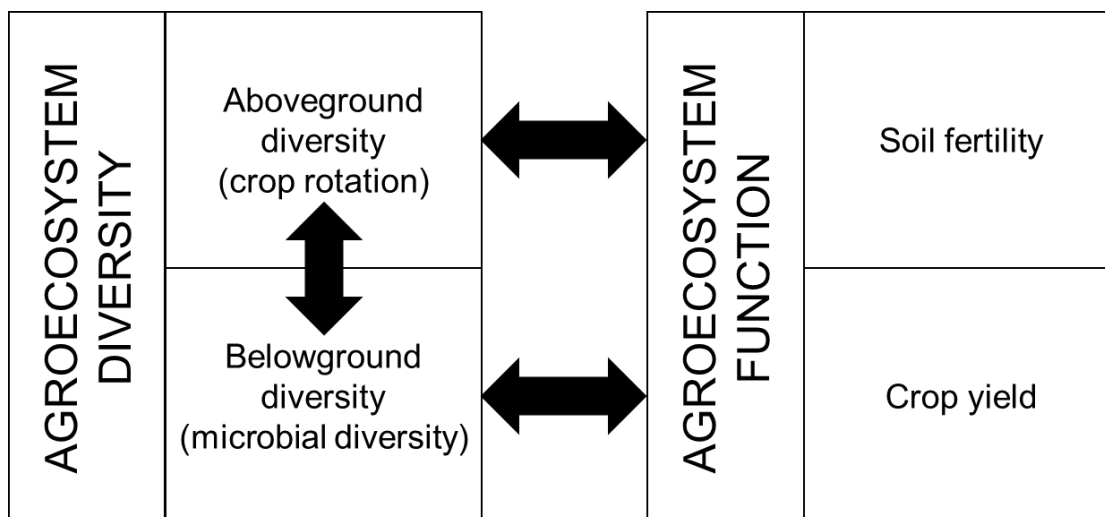


Figure 1.2: Theoretical framework for the research aims of the present thesis. Research aims are focused on relationships indicated by arrows.

Chapter 1 of the thesis will address these aims broadly through a quantitative assessment of the effect of increased crop diversity on soil microbial diversity and agroecosystem function in terms of soil N fertility. Meta-analysis is used as a tool to look at a broad range of crop rotation studies from around the world.

Chapter 2 focuses on wheat-legume crop rotations in the Swartland Local Municipality of South Africa. Data collected from soils and crop under a 19-year wheat-legume crop rotation trial will be assessed according to the theoretical framework (Figure 1.2). It is important to note that the initial study design for Chapter 2 involved the sampling of *Rhizobium* diversity in legume rotations, as well as an isotope analysis of $\delta^{15}\text{N}$ abundance in the fertilizer, sheep dung and plant material. The results from this data would assist in better understanding the ecosystem-diversity function relationship by assessing the N cycling within the agroecosystem, more specifically, by estimating the proportion of plant N sourced from microorganisms. Unfortunately, miscommunication with laboratory staff led to the destruction of our sample material and these analyses could not be carried out.

Throughout the thesis, results will be discussed in terms of the broader diversity-ecosystem function relationships (Figure 1.1) and the theoretical framework (Figure 1.2). Understanding the relationship between agroecosystem diversity and function by testing if it aligns with broader ecosystem theories may assist in identifying more sustainable agricultural management practices that enhance agroecosystem function both in terms of yield and ecosystem services. It is clear that the causal mechanisms through which crop-legume or crop-non legume rotations increase wheat production and economic viability are not fully understood. Further insight into beneficial soil ecological factors associated with wheat-

legume rotations can benefit the wide-spread adoption of conservation agriculture within the Western Cape of South Africa.

CHAPTER 2: THE IMPACT OF CROP ROTATION ON SOIL MICROBIAL DIVERSITY AND NITROGEN FERTILITY: A META-ANALYSIS

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Abstract

Agricultural intensification can involve the simplification of agroecosystems to crop monocultures while the practices of crop rotation, intercropping and companion planting maintain some crop diversity over time and space, respectively. It is generally postulated that reduced diversity can have an impact on ecosystem function. Here we determine whether decreased aboveground crop diversity affects belowground microbial biodiversity and associated nitrogen fertility by conducting a meta-analysis of studies comparing monocultures and crop rotations. Using 26 and 43 individual weighted mean differences, we found that soils under a higher diversity of crops in rotation produced higher microbial richness (+15.11%) and diversity (+3.36%) scores, respectively. This effect was significantly influenced by microbial analysis method where pyrosequencing produced conflicting results to those from fingerprinting methods. Longer study trials with lower annual percentage ground cover and no legumes produced larger increases in microbial diversity. No correlation between microbial diversity and soil nitrogen fertility was found. This provides support for the hypothesis that ecosystem function may be a product of either specific productive species (selection effect), or the facilitative interaction of multiple species (complementarity effect). It is plausible that productive agroecosystems may have high and/or low microbial diversity. Although there is a small positive effect of crop rotation on microbial diversity, the link between diversity and agroecosystem function remains complicated. A lack of studies incorporating next-generation sequencing techniques to elucidate complex microbial community structures and specific functional niches in crop rotational agroecosystems highlights scope for future research.

Key Words:

Agroecosystem; crop rotation; meta-analysis; microbial diversity; nitrogen fertility

2.1. Introduction

The intensification of conventional agricultural practices is threatening ecosystem services and agroecosystem sustainability through soil erosion, agro-chemical pollution of groundwater, release of green-house gases and biodiversity loss (Tilman, 2001). This is causing a paradigm shift toward sustainability, characterized by practices and concepts such as organic agriculture (Badgley et al., 2007), conservation agriculture (Hobbs et al., 2008), agroecology (Rosset and Altieri, 1997; Thomas and Kevan, 1993) and functional agrobiodiversity (Wood and Lenne, 1999). One of the important management practices associated with sustainability, which precedes the green revolution, is crop rotation. Before the introduction of petrochemicals (pesticides, herbicides and fertilizers), the means by which soil fertility was maintained and pests were managed was through the diversification of crops over time and in space on the same piece of land (Karlen et al., 1994).

The historical adoption of crop rotations was largely motivated by the associated yield increase in the cash crop (Bullock et al., 1992). The correlation between yield increase and increasing crop diversity has been shown through numerous scientific studies and has been attributed to enhanced agroecosystem function (Smith et al., 2008). The causal mechanisms through which this yield increase is achieved include increased soil fertility (particularly when leguminous plants are used in rotation), maintenance of soil structure, disruption of pest cycles and weed suppression. These processes are mediated largely by soil microorganisms which play an essential role in sustaining productive agroecosystems through their complex biochemical processes (Kennedy and Smith, 1995; Kennedy, 1999; Parkinson and Coleman, 1991). Microbial communities within soils are more diverse and numerous than higher-order organisms and the full extent of this biodiversity is still unknown (Schmidt et al., 2015; Torsvik et al., 1990). The primary drivers of microbial diversity include edaphic variables such as soil pH and moisture, yet plant diversity is also now recognised as an important driver of soil microbial diversity (Berg and Smalla, 2009).

Given that plant diversity is a primary driver of microbial diversity, it has been posited that management practices such as crop rotation, through increasing aboveground biodiversity, can result in corresponding increases in diversity belowground (Hooper et al., 2000). Different crops have associated root exudates of varying composition, which result in plant-specific effects on the soil microbial communities in the rhizosphere (Costa et al., 2006; Wardle et al., 2004). Rhizosphere communities can have an impact on the bulk soil microbiome (Kent and Triplett, 2002). In addition, crop rotations lead to a greater abundance and diversity of plant litter, which in turn can support a greater diversity of microbial decomposers (Kennedy, 1999). Thus increases in microbial diversity may not be due to increased plant diversity per se, but

associated increases in soil organic matter caused by increased ground cover in rotations (Zak et al., 2003).

This increase in microbial diversity as a result of incorporating a variety of crops in rotation has been shown. For example, Lupwayi et al. (1998) discovered that microbial diversity, based on community level substrate utilisation, was significantly higher under a rotation of wheat and clover or field peas than under continuous wheat. However, other studies have shown that crop rotation has limited or negative effects on microbial diversity. For example, Navarro-Noya et al. (2013) found that crop management (continuous maize versus maize-wheat rotation) had no effect on soil microbial diversity. Yin et al. (2010) found that incorporating soy beans into rotation with wheat decreased richness and Shannon's diversity indices in conventional tilled plots when compared to continuous wheat. Thus, there is conflicting evidence in the literature addressing the relationship between above and belowground biodiversity in agroecosystems.

Furthermore, it is difficult to elucidate the relationships between biodiversity (above or belowground) and agroecosystem function as they exist within a net-like causal structure with non-linear interactions. However, advances in molecular biology such as next-generation sequencing (NGS) are allowing for accurate and efficient analyses of complex microbial communities and their niche functions (Fakruddin et al., 2013). This allows for the analysis of the relationship between crop diversity, functional microbial diversity and ecosystem services in the soil. In the current literature there are examples of microbial diversity and agroecosystem function being both coupled and decoupled (van der Heijden and Wagg, 2013; Wagg et al., 2014; Welbaum, 2004). In addition, the extent to which the diversity function relationship varies with temporal (e.g. season) and spatial factors is unclear (van der Putten et al., 2009).

Within the broader context of diversity-ecosystem function theory it is possible that an increase in plant species number within a crop rotation system results in a coupled increase in agroecosystem function (Smith et al., 2008; Vitousek and Hooper, 1994). However, the mechanism behind this positive effect may be attributed to singular key-stone species such as N₂-fixing legumes (the selection effect) or due to the facilitation and niche differentiation associated with a number of species (the complementarity effect) (Hooper et al., 2000). It is often the case that both these mechanisms are at work which gives support for the decoupling and coupling of biodiversity with function.

Another theory suggests the diversity of microorganisms within soil can contribute toward agroecosystem resilience to disturbance or stress through functional redundancy (Giller et al., 1997). Experiments have shown that higher microbial diversity results in shorter recovery time

in pasture soil communities after short-term and long-term stress, i.e. increased resilience (Griffiths et al., 2000). The diversity of endophytic and rhizospheric plant growth-promoting microorganisms act as antagonists to plant pathogens and increase tolerance to salinity, drought, temperature and nutrient deficiency (Dias et al., 2015). Thus, high microbial diversity acts as an insurance against ecosystem malfunctioning due to multiple taxonomic sub-units that can perform the same function and have variable tolerances to stress. Yet, for the same reason microbial diversity and soil ecosystem function can be largely decoupled in microbial communities.

Evidence also suggests that high microbial diversity is linked to increased nutrient and water use efficiency in soil (Brussaard et al., 2007). It is well-known that soil microbes enhance soil fertility through the mineralisation of limiting nutrients such as nitrogen (N) and phosphorus (P) (Dias et al., 2015). For example, N₂-fixing bacteria and mycorrhizal fungi are responsible for 5 to 20% N and 75% of P acquired in grassland and savannah plants annually (van der Heijden et al., 2008) as well as in the crop plants wheat and carrot (Hawkins et al., 2000). In agricultural systems, there are examples of the association between high microbial diversity and higher soil N content for crops grown in rotation (Marinari et al., 2015; Murphy et al., 2011), although a systematic review of such studies has not yet been performed.

A quantitative assessment of the literature on the effect of increased crop diversity on soil microbial diversity and agroecosystem function in terms of soil N fertility, a major limiting element in crop productivity, has not previously been performed. Meta-analysis was used as a review tool to quantify this effect. This method measures the impact of a given experimental treatment relative to a control based on the research results from multiple independent studies (Hedges et al., 1999). Although originally developed for medical and social science reviews, meta-analysis has been adapted for application to many ecological and agricultural datasets in the literature (e.g., Johnson and Curtis, 2001; McDaniel et al., 2014; Tonitto et al., 2006). This technique is used to answer the following two questions: (1) Does increasing diversity of crops in rotation result in increased soil microbial diversity, and (2) does increased soil microbial diversity result in increased soil N fertility?

2.2. Methods and materials

2.2.1. Meta-analysis criteria

As a tool, meta-analysis allows for the quantifying of an effect across broad geographical regions including experimental trials of varying lengths, methodologies and crop combinations. The analysis requires that studies comprise of experimental treatments that are compared to

a control that can be defined consistently across studies. For the purposes of this analysis, the control was defined as any annual cash crop that is grown in monoculture every season. The experimental treatment/s were defined as any crop rotation including the same cash crop and at least one other rotation crop. Studies were included that recorded taxonomic or functional measures of soil (free-living) microbial diversity, including the Shannon's diversity index (Zak et al., 1994) and/or richness. These are the most commonly used measures of soil microbial diversity in agricultural studies and are often used interchangeably without qualification or definition (Spellerberg and Fedor, 2003). For this analysis, species richness is defined as the number of unique taxonomic sub-units, and diversity as the richness and relative abundance of these sub-units. The inclusion of a measure of soil N fertility was not part of the selection criteria, but was analysed for association to microbial diversity post hoc. Many studies were excluded because they did not (1) contain the control, experimental treatment/s and diversity measures as listed above, (2) contain purely agricultural cash crops, or (3) metadata was not available upon request. From the rejected studies, those that reported some measure of microbial diversity were collated with accepted studies according to whether they detected significant increase, decrease or no change in microbial diversity with an increase in crop diversity over time.

2.2.2. Literature search and data extraction

The literature was searched using electronic databases, including GoogleScholar, AGRIS, ScienceDirect, Elsevier and Wiley by entering the following search terms used in various combinations: crop rotation, microbial diversity, microbial richness, function, nutrient cycling, nitrogen fertility. The Boolean operators 'AND' and 'OR' were used to combine two separate searches and include alternative search terms respectively. We initially used the broad search string 'crop rotation AND microbial diversity OR microbial richness'. Following this, we added 'OR function', 'OR nutrient cycling', and 'OR nitrogen fertility' to the initial search string respectively. The following criteria were used to select studies: 1) only peer reviewed articles in journals; and 2) studies with sufficient sample size to determine both a mean and standard error. In addition, studies were also identified through a 'snowballing' technique where reference lists of acquired studies were searched for additional relevant studies. For some studies, Data Thief® (Tummers, 2006) software was used to extract values from figures within studies. In cases where diversity measures or variances were not reported, the corresponding authors were contacted to request this information. Where this information was forthcoming it was included in the analysis.

A total of 20 studies met the selection criteria and were entered into a database containing the following categorical moderating variables: microbial analysis method (biochemical

fingerprinting, molecular fingerprinting and pyrosequencing), number of crops in rotation (2 or 3), use of legume (yes or no), use of cover crop (yes or no), percentage of year with ground covered by crop (~50% or ~100%), and trial length (1-5, 6-15 and >15 years) (Appendix A). Here cover crop is defined as a crop that is not harvested but produced to enrich some aspect of soil health. To account for differences between NGS and other microbial analysis methods, studies using biochemical fingerprinting [fatty acid methyl ester (FAME) analysis, community-level physiological profiling (CLPP) and plate enumeration], molecular fingerprinting [denaturing gradient gel electrophoresis (DGGE), random amplified polymorphic DNA (RAPD) analysis, restriction fragment length polymorphism (RFLP)], and pyrosequencing were separated. Differences in categorical moderating variables were analysed using ANOVA.

From the selected studies, 43 individual Shannon diversity index comparisons and 26 individual richness comparisons were obtained. From these, eight individual associations between diversity and a functional measure (soil N content) were extracted. These studies focused on soil N fertility and did not include measures of N uptake in plants. Many studies applied multiple treatments with different combinations of crops in rotation at varying lengths of time. We treated each unique rotation combination and rotation length as independent observations. In addition, where within-treatment effects such as tillage (e.g. Yin et al., 2010), fertilization (e.g. Reardon et al., 2014), length of rotation (Bucher and Lanyon, 2005), or analysis method (Yao et al., 2006) were recorded in split-plot designs, these factors were recorded as independent observations if they were significantly different. In cases where they were not significantly different they were averaged and entered as one observation.

2.2.3. *The Quality Effects Model*

The meta-analysis of studies with usable data that investigated the effect of increasing crop diversity on soil microbial diversity and soil fertility was performed using the Quality Effects Model (Doi and Thalib, 2008) in MetaXL (v. 2.2, Epigear International). In conducting the meta-analysis, an effect size estimate was calculated for each of the measurable variables to quantify the magnitude of the treatment effect (Osenberg et al., 1999). The effect size estimate used was the weighted mean difference (WMD), which is the difference in the mean response between the treatment (\bar{X}_t) and the control (\bar{X}_c). We calculated this as a percentage difference ($\frac{\bar{X}_t - \bar{X}_c}{\bar{X}_c} \cdot 100$) so that positive values indicated an increase in microbial diversity or soil N with crop rotation/crop diversity relative to controls/monocultures. Within MetaXL, the quality effects model was used with effect size estimates weighted by variance, at 95% confidence intervals, as well as a quality score (Qi). If the 95% confidence intervals of effect sizes did not overlap with zero or other treatments, they were considered significant at $P < 0.05$ (Johnson and Curtis, 2001). The Qi estimates the likelihood that results of a study are unbiased due to

possible flaws in the experimental design (Doi and Thalib, 2008). The heterogeneity in the credibility of studies included in the meta-analysis is important to quantify because if the quality of an experimental trial is inadequate, it may falsify the conclusions of the review. The Qi is calculated based on selected criteria with weighted scores between 0 and 1 representing low and high quality respectively (Table 2.1). An additional measure of between-study heterogeneity, I² statistic (percentage variation across studies that is due to heterogeneity rather than chance), was also generated by MetaXL (Higgins et al., 2003).

A few studies (7/20 studies) did not report any measures of variance and metadata could not be obtained upon request. Thus the within-study variances that were available were used to calculate a pooled variance for the set of studies. This was used to calculate confidence intervals for studies lacking variance under the assumption that the variances of the responses within these studies were homogenous. The studies which did not calculate variance were not discriminated against in the scoring of Qi.

Table 2.1: Quality scoring system used in the Quality Effects model of the meta-analysis to assess the study design and possible bias. Questions and scores are adapted from epidemiology (Doi and Thalib, 2008) to be relevant to agronomy.

Question	Score
1. Did the experimental layout use randomization or another appropriate sampling strategy?	0 = No or not reported 0.5 = In part 1 = Yes
2. Were the groups being compared comparable at the baseline?	0 = No or not reported 0.5 = In part 1 = Yes
3. Were treatments clear and not confounded by e.g. soil type, cultivation history, tillage?	0 = No 0.5 = In part 1 = Yes
4. Was the trial conducted over an adequate time period to allow differences to emerge?	0 = 1-5 years 0.5 = 6-10 years 1 = 11-20 years 2 = >20 years
5. Was the analysis clearly reported and appropriate?	0 = No 0.5 = In part 1 = Yes
6. Were protocol deviations or losses during the study acceptable (<20%)	0 = No or not reported 0.5 = In part 1 = Yes
Quality Score (Qi) = $\frac{\text{Sum of scores}}{7}$	

2.3. Results

Of the hundreds of studies screened through the database searches, only 27 reported some measure of microbial diversity in response to a change in crop diversity. Overall, there was a

net positive effect of rotation on microbial diversity, although 14 studies did not report any significant changes (Figure 2.1). Of the initial 27 studies, 7 had to be rejected for meta-analysis because they did not (1) report Shannon's diversity index or richness scores (Chen et al., 2012; Ferreira et al., 2000; Larkin et al., 2008; Li et al., 2010; Souza et al., 2013), or (2) increase crop diversity over time (Dorr de Quadros et al., 2012), or (3) measure free-living microbial diversity (Herrmann et al., 2014).

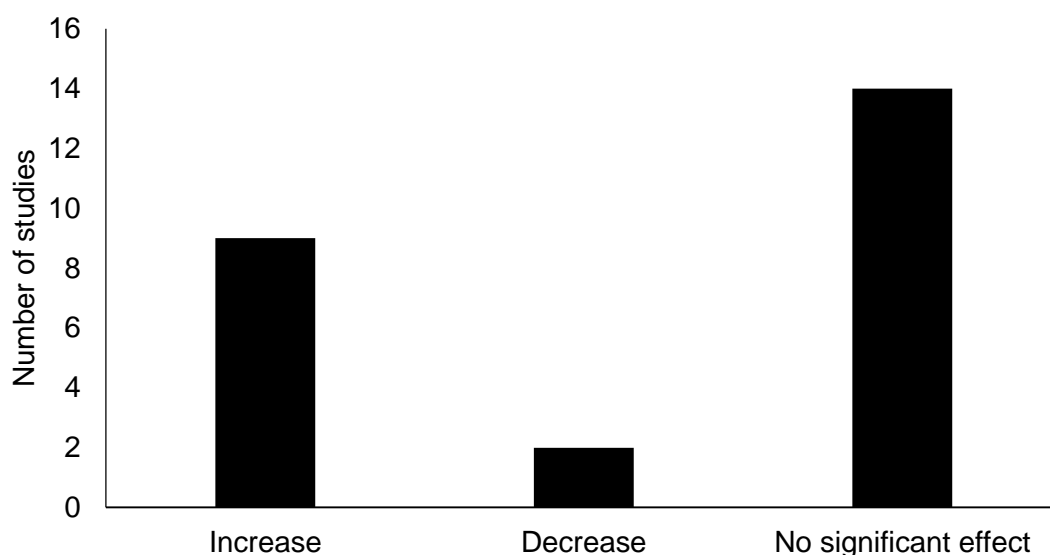


Figure 2.1: Number of studies reporting soil microbial diversity (including studies that did not meet the quality criteria for data extraction) that show a significant increase, decrease or no significant effect in soil microbial diversity with an increase in crop diversity.

2.3.1. Diversity and richness

There were 17 studies retrieved that measured the effect of increasing crop diversity on soil microbial diversity using Shannon's diversity index (H') and from these, 43 individual WMDs were calculated. The meta-analysis revealed that there was on average an increase of 3.36% in microbial H' with increasing crop diversity ($p < 0.05$, Figure 2.2). There were 13 studies retrieved that measured the effect of increasing crop diversity on soil microbial richness and from these, 26 individual WMDs were calculated. The meta-analysis revealed that increasing crop diversity increased soil microbial richness on average by 15.11% ($p < 0.05$, Figure 2.3). However, both forest plots for microbial H' and richness revealed moderate levels of heterogeneity with an I^2 of 35% and 45% respectively (Higgins et al., 2013).

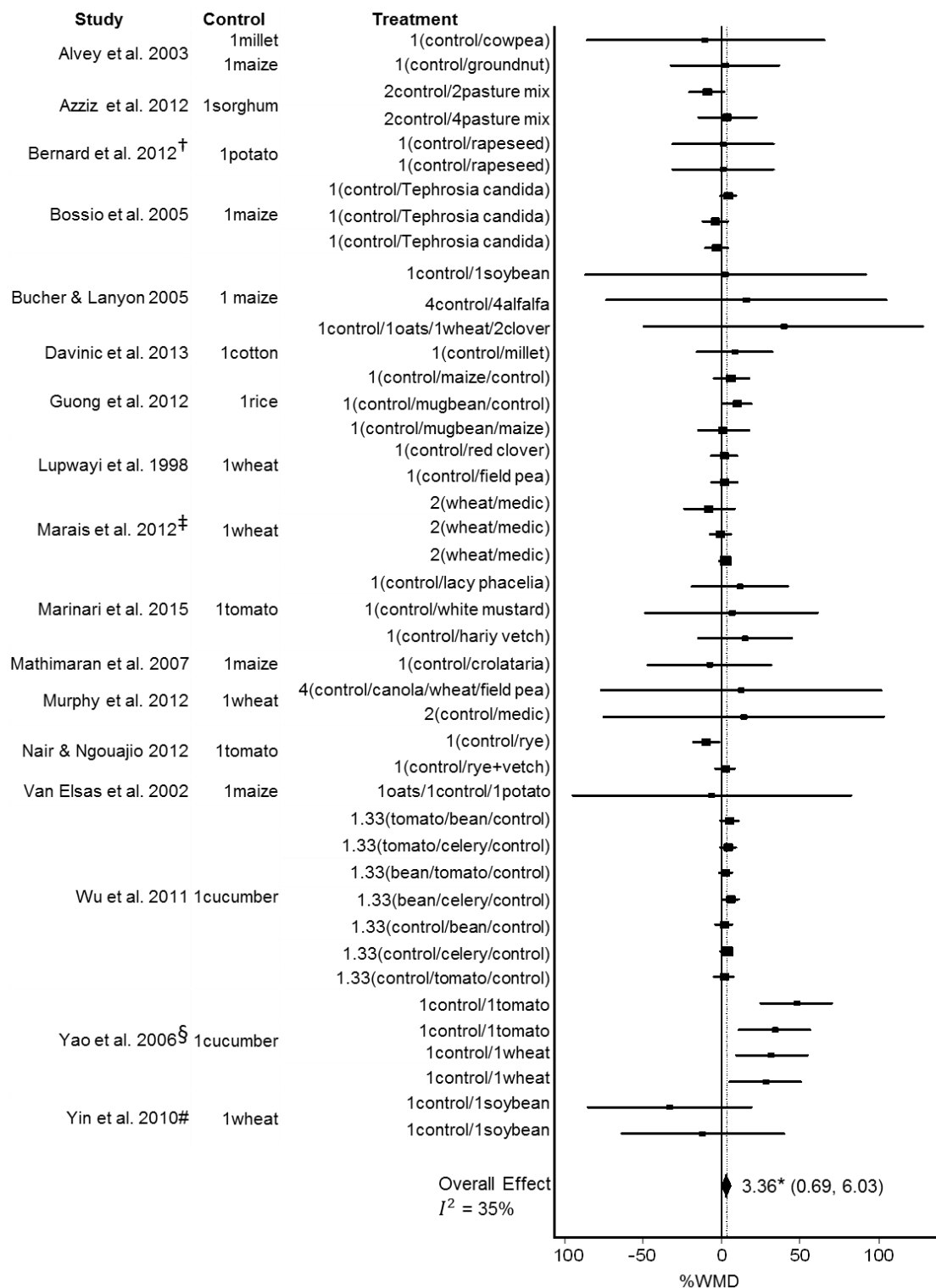


Figure 2.2: Forest plot of percentage weighted mean difference (WMD) in soil microbial diversity (H') between crop monocultures (1 crop) and crop rotations (>1 crop) from 17 studies using the WMD method and Quality Effects model (Doi and Thalib 2008; 2009) in MetaXL (v. 2.0, Epigear International). Studies are shown on the left with control crop and treatment combinations listed with numbers signifying number of years. The Forest plot is shown on the right where studies >0 and <0 showed a positive or negative treatment effect, respectively. Treatment effect indicated with squares, which are

weighted according to the weight (%) that the study was given based on the quality score (Table 2.1). Confidence intervals are indicated by horizontal lines. Overall effect size displayed as a diamond where the width thereof indicates 95% confidence intervals. Where these confidence intervals cross the 'line of no effect' (midline) the overall result indicates non-significance at the $P = 0.05$ level. I^2 measures between-study heterogeneity. *Overall effect size with confidence intervals in parenthesis. †Treatments separated because of two study sites. ‡Treatments separated based on year. §Treatments separated based on analysis method (CLPP/RAPD). #Treatments separated based on tillage (till/no-till).

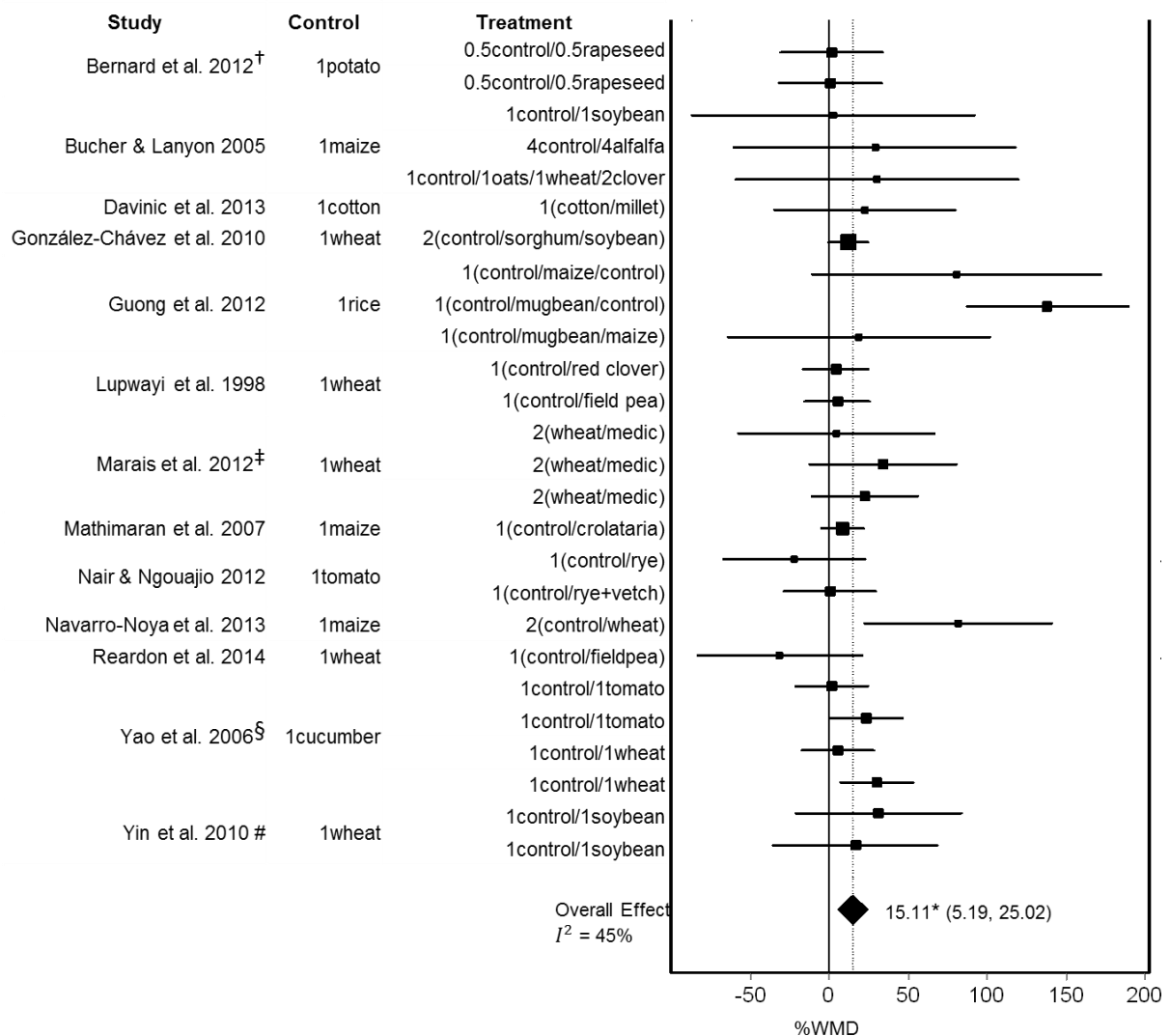


Figure 2.3: Forest plot of percentage weighted mean difference (WMD) in soil microbial richness between crop monocultures (1 crop) and crop rotations (>1 crop) from 13 studies using the weighted mean difference (WMD) method and Quality Effects model (Doi and Thalib 2008; 2009) in MetaXL (v. 2.0, Epigear International). Studies are shown on the left with control crop and treatment combinations listed with numbers signifying number of years. The Forest plot is shown on the right where studies >0 and <0 showed a positive or negative treatment effect, respectively. Treatment effects are indicated with squares, which are weighted according to the weight (%) that the study was given based on the

quality score (Table 2.1). Confidence intervals are indicated by horizontal lines. Overall effect size displayed as a diamond where the width thereof indicates 95% confidence intervals. Where these confidence intervals cross the 'line of no effect' (midline) the overall result indicates non-significance at the $P = 0.05$ level. I^2 measures between-study heterogeneity. *Overall effect size with confidence intervals in parenthesis. †Treatments separated because of two study sites. ‡Treatments separated based on year. §Treatments separated based on analysis method (CLPP/RAPD). #Treatments separated based on tillage (till/no-till).

For studies which reported both diversity (H') and richness scores, a regression analysis of WMD richness on WMD H' scores was performed. There is a no significant relationship ($R = 0.063$, $p = 0.776$) between the change in diversity and change in richness after increasing crop diversity.

2.3.2. *Categorical moderating variables*

Studies that used pyrosequencing as an analysis method measured an average decrease in H' scores with rotations and this was significantly different to biochemical and molecular fingerprinting methods, which revealed increases in H' scores ($p = 0.047$, Table 2.2). The opposite trend was true for richness scores, as pyrosequencing studies revealed a significantly higher increase in microbial richness with rotations compared to that found by studies using fingerprinting methods ($p = 0.002$). Longer study trials (>6 years) contained larger increases in microbial richness with rotations ($p = 0.010$) and a similar trend was observed for diversity. Significantly higher increases in diversity was observed in studies with no legumes ($p = 0.043$) and shorter ground cover (50% of the year; $p = 0.006$). The same trend was observed for studies including no legumes, lower percentage ground cover and higher number of crops (Table 2.2).

Table 2.2: Number of observations (K), mean size effect and ANOVA P values for Shannon's H' diversity and richness scores for each of the categorical moderating variables.

Moderating Variable	Categories (levels)	Diversity			Richness		
		K	Mean size effect	P	K	Mean size effect	P
Analysis method	Biochemical fingerprinting	21	12.35 ± 3.38	0.047*	15	10.5 ± 3.83	0.002**
	Molecular fingerprinting	19	7.52 ± 3.64		5	5.85 ± 10.68	
	Pyrosequencing	7	-4.98 ± 5.52		6	61.27 ± 15.47	
No. crops in rotation	2 crops	30	6.6 ± 1.1	0.703	19	24.5 ± 8.53	0.582
	3 crops	9	4.82 ± 0.65		5	6.77 ± 3.76	
Legume	Yes	25	2.03 ± 2.45	0.043*	15	20.52 ± 9.1	0.883
	No	15	11.28 ± 4.24		10	22.61 ± 10.85	
Cover crop	Yes	25	1.75 ± 1.26	0.058	13	16.05 ± 11.29	0.443
	No	18	10.13 ± 0.68		13	26.59 ± 7.46	
Ground cover [†]	50%	12	14.6 ± 6.79	0.006**	10	25.24 ± 7.31	0.654
	100%	31	1.64 ± 1.1		16	18.87 ± 10.04	
Trial length	0-5 years	25	2.88 ± 1.33	0.450	9	-0.146 ± 5.32	0.01*
	6-15 years	8	8.7 ± 5.08		6	50.07 ± 20.57	
	>15 years	10	8.46 ± 8.04		11	24.28 ± 6.61	

Notes: * and ** represents significance at $p < 0.05$ and $p < 0.01$, respectively. [†] Rotations including two or more crops per year were considered as 100% and rotations including one crop per year were considered as 50%.

2.3.3. Soil N fertility

From the total study database, four studies recorded some measure of soil fertility (total soil N) associated with the changes in soil microbial communities in response to the rotation treatments. None of the studies reported any measure of variance for the soil N content, thus a weighted meta-analysis could not be performed on this variable. Instead, a regression analysis of the percent change in microbial diversity (H') on the percent change in soil N was performed. The relationship between soil N content and microbial diversity was not significant ($R = 0.0553$, $p = 0.575$).

2.4. Discussion

The goal of this meta-analysis was to test the hypothesis that increasing crop diversity results in increased soil microbial diversity and agroecosystem function in terms of soil fertility according to the ecological biodiversity stability relationship (Vitousek and Hooper, 1994). More specifically, it has been suggested that increasing aboveground biodiversity can result in a proportional increase in belowground biodiversity (Hooper et al., 2000), and that soil

microbial diversity can enhance agroecosystem functioning (van der Heijden and Wagg, 2013; Welbaum, 2004). Thus it was predicted that increasing crop diversity would result in an increase in soil microbial diversity and richness as well as soil N content. Shannon's diversity index and richness of soil microorganisms as well as measures of soil N content were used as comparable measures between studies.

2.4.1. Crop diversity and soil microbial diversity

The overall effect sizes indicate that increasing crop diversity has a positive effect on soil microbial diversity and richness. The results from the meta-analysis confirms what many other broader ecological studies report about positive relationships between above and belowground biodiversity (Hooper et al., 2005; Zak et al., 2003). This trend has also been observed for soil macrofauna. Sileshi et al. (2008) found an increase in soil macrofaunal richness and abundance in maize-legume rotations when compared to continuously cropped maize.

The causal mechanisms behind this increase in belowground microbial diversity could involve physico-chemical changes in the soil brought about by increased crop diversity (Dias et al., 2015). Crop rotations are known to influence the physical structure of the soil and enhance soil water-use efficiency and temperature stability through increased ground cover and soil organic matter content (Kennedy, 1999). The differential root action and niche exploitation from successive crops can allow for the proliferation of microbes to a larger extent in the bulk soil. These physical changes can create a favorable microclimate for soil microbes to thrive. The chemical changes in soil mediated by rotations are caused predominantly by build-up of residual root exudates and plant litter from preceding crops (Garbeva et al., 2004). These provide a greater diversity of residual carbon (C) substrates in the bulk soil which can support the growth of diverse microorganisms. Other studies have shown the host-specificity of bacterial and fungal groups in agricultural systems (Berg and Smalla, 2009; Smalla et al., 2001; Wardle et al., 2004). For example, Costa et al. (2006) detected plant specificity in the rhizosphere by bacterial, fungal and group-specific denaturing DGGE profiles for strawberry and oilseed rape crop. These plant-specific microbial species are predominantly of soil origin, as studies have shown that rhizosphere communities are more similar to bulk soil communities than to endophytic communities (Kent and Triplett, 2002). Thus, rather than introducing new species into the soil system, rotation crops stimulate the growth of specific microbial communities that are latent in the bulk soil. These residual rhizosphere communities from antecedent crop can have an impact on the bulk soil microbiome.

Thus, changes in microbial communities may not be due to increased plant diversity per se. Zak et al. (2003) found that microbial communities responded to increased plant production of

detritus and C substrates associated with higher plant diversity rather than to the diversity itself. In addition, adding legumes to a rotation has been shown to increase bulk soil C pools, supporting a greater abundance of microbiota (Carranca et al., 2009; Drinkwater et al., 1998). Leguminous plants also harbor host-specific symbiotic bacteria (rhizobia) which cannot exist without their hosts. These physico-chemical factors can have positive feedbacks into microbial growth and abundance and, as the meta-analysis results suggest, even diversity.

Despite the overall positive effect of rotation on microbial diversity and richness, there were outlier studies which revealed negative relationships. Yin et al. (2010) found that wheat in rotation with soybean resulted in a decrease in microbial diversity when compared to continuous wheat (Figure 3.2). This may have been caused by the rise in pH associated with leguminous rotations which may negatively affect microbial diversity. Another study on West African soils also found that grain-legume rotations increase pH in the bulk and rhizosphere soils (Alvey et al., 2001). Reardon et al. (2014) found that wheat in rotation with field pea negatively affected microbial richness (Figure 2.3). This value exhibited large error margins which may have been attributed to year-to-year variability in the richness scores possibly driven by climatic variables. There was no common trend in crop type among studies exhibiting negative relationships to suggest host specificity.

The presence of the outlier studies is evidence of the large heterogeneity between and within studies. Further, meta-analysis can be limited by publication biases, inherent problems in the design of studies, and the subjectivity related to pooling similar studies (Garg et al., 2008). However, within the present study, these were accounted for through the use of the Quality Effects Model (Doi and Thalib, 2008). Heterogeneity between studies in terms of study scope and methodology was accounted for by analysing data using categorical moderating variables. Meta-analysis is an important empirical tool that can assist in the critical review of literature by quantifying trends from a large number of articles in an objective manner.

2.4.2. Microbial diversity vs. richness

Although there is an overall increase in both diversity and richness with crop diversity, the regression analysis reveals that a positive linear relationship between richness and diversity is not necessarily the case. For example, Guong et al. (2012) and Davinic et al. (2013) produced comparable WMDs in H' (9.91% and 8.75%, respectively), yet had disproportionate WMDs in richness (138.02% and 22.73% respectively). This aligns with other broad ecological studies which have shown that species richness and evenness were uncorrelated for different taxonomic groups in an American savannah (Bock et al., 2007). To understand this relationship it is necessary to note that Shannon's diversity H' incorporates both alpha (richness) and beta (evenness) diversity as it takes into account the proportional abundance

and distribution of different taxonomic sub-units (Peet, 1974). Thus a given increase in the H' value can represent a population shift to higher richness and lower evenness (which may be the case with Guong et al., 2012), or a shift to lower richness and higher evenness (which may be the case with Davinic et al., 2013). Measures of alpha diversity such as Shannon's H' are considered more reliable estimates of diversity in terms of ecosystem functioning because measuring richness alone can ignore species that are disproportionately abundant, for instance pathogens (Hill, 1973). The results of the meta-analysis confirm that diversity and richness are not always positively correlated.

2.4.3. Categorical moderating variables

Results from pyrosequencing studies differed to those from fingerprinting studies for both richness and diversity scores. This may be due to the higher accuracy of NGS compared with fingerprinting methods, or due to other study-specific variables. Pyrosequencing revealed an average decrease in microbial diversity in response to rotations, yet an increase in richness. This suggests that although new species may emerge with crop rotations, the relative abundance of microorganisms within species remains homogenous between species. However, the low number of studies using pyrosequencing to assess crop rotation may explain this heterogeneity and highlight the need for further application of NGS within agroecological studies.

The results also revealed a tendency toward greater increase in microbial diversity and richness in study trials spanning greater lengths of time. This has been shown in an ecological grassland experiment where the effect of plant diversity on soil microorganisms was most pronounced after a lag period of four years (Eisenhauer et al., 2010). A number of studies in the meta-analysis reported a decrease in the response of microbial diversity to crop rotations after harvest (Guong et al., 2012; Marinari et al., 2015). These studies were based on trials shorter than ten years. It could be hypothesized that there is an accumulated increase in the residual effect of rotation on soil microbial diversity over time spans exceeding ten years of the same treatment through increased soil C.

The tendency toward decreased microbial diversity and richness associated with longer ground cover and presence of legumes and cover crops in rotation is surprising. Many studies show that incorporating legumes and cover crops into rotations can boost soil C and thus support greater microbial abundance and diversity (McDaniel et al., 2014). Longer periods of ground cover enhance soil microclimates and are thus expected to support microbial growth (Kennedy, 1999). However, other studies have also shown that key functional plant groups like grasses and legumes have inconsistent effects on soil microbial functioning and diversity (Eisenhauer et al., 2010).

2.4.3. Crop diversity and soil N fertility

There was no significant correlation between the percentage change in soil microbial diversity and the percentage change in soil N content. The strength of this correlation might be increased if a larger number of studies that reported soil fertility in combination with microbial diversity and richness were available. Alternatively, the weak correlation may reflect that one soil element cannot be expected to be functionally related to a complex system with various ecological functions.

Although all studies showed an increase in soil N with increasing crop diversity, the question is whether this increase was mediated by an associated increase in microbial diversity or crop residue quality or quantity. In a long-term ecological trial on grassland and savanna vegetation, increasing plant diversity (1-16 species) was positively correlated with more rapid N mineralization (Zak et al., 2003). There was also an associated increase in microbial biomass and composition which suggests that plant-microbe interactions are integral in the link between plant diversity and ecosystem function. In an agricultural meta-analysis, McDaniel et al. (2014) found that increasing crop diversity in rotation, increased soil C and N by 3.6% and 5.3%, respectively, but when a legume cover crop was included in rotation, these values increased to 8.5% and 12.8% (i.e. the addition of one plant more than doubles N and C values). This suggests that the functioning of the soil fertility in agroecosystems is largely mediated by particular, host-specific and productive genera (e.g. *Rhizobia* spp.). Thus a productive system may well be low in microbial diversity. This is known as the selection effect and is different to the complementarity effect where the positive effect of biodiversity is achieved through niche differentiation and facilitative interaction (Hooper et al., 2000). It is possible that microbial diversity drives soil fertility and thus plant growth through both selection and complementarity effects. For example, the diversity of belowground plant-associated soil fungi has been shown to improve plant productivity through the selection and complementarity effects by up to 82% and 85%, respectively (Wagg et al., 2011). Thus it is clear that, as indicated by our results and other literature, increased microbial diversity can facilitate greater agroecosystem functioning through enhanced soil fertility but that this relationship is sometimes uncoupled and is likely context specific. The future use of NGS and other methods that link taxonomic units to their ecological functional niches within agroecosystems may elicit further clarity.

2.5. Conclusions

A review of the literature and a meta-analysis of the data therein showed that increasing the diversity of crops in rotation has a positive impact on soil microbial richness (+15.11%) and

diversity (+3.36%). However, this effect was highly variable and suggests that the link between above and below ground diversity is related to specific functional groups. Furthermore this effect was not significantly influenced by the number of crops, presence of legumes or cover crops in rotation, percentage of year with ground under living crop, or trial length. Microbial diversity was not significantly correlated with soil N fertility, although a larger sample size may clarify such correlation. Our results show that, even though it is clear that crop rotation is beneficial for soil quality and consequently crop yield, the link between diversity and function remains unclear. Thus adopting crop rotations may increase soil microbial diversity, but it is not clear whether this will enhance soil N fertility. Future research on the link between above and belowground diversity would do well to measure the associated effects on components of agroecosystem function (e.g. soil N fertility) as well as the spatial and temporal changes therein. In addition, the use of NGS techniques can aid in understanding the role of specific functional groups in the yield increase of crop rotation systems.

CHAPTER 3: THE IMPACT OF WHEAT-LEGUME CROP ROTATIONS ON SOIL MICROBIAL DIVERSITY AND AGROECOSYSTEM FUNCTION IN THE SWARTLAND OF SOUTH AFRICA

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Abstract

Conservation agriculture incorporates crop rotations as a means of enhancing ecosystem services within agroecosystems, which promote their sustainability. This diversification of aboveground crops through crop rotations and the resultant changes in rhizosphere biochemistry and niche availability can have impacts on belowground microbial communities, which are responsible for the bulk of ecosystem processes and services in soils. It is known that crop rotation with legumes increases microbial biomass, however little is known about the effects on microbial diversity and function. We hypothesize that increased crop diversity that includes legumes will result in increased microbial diversity and hence nutrient cycling, specifically of N. Community level physiological profiling (CLPP) and automated rRNA intergenic spacer analysis (ARISA) were used as measures of functional and genetic microbial diversity, respectively, in soils of a 19-year wheat-legume crop rotation trial in the Swartland Local Municipality of South Africa. Increasing crop diversity through rotations of wheat with medic (Wm) or a combination of medic and clover (Wmc) resulted in greater wheat plant stem length and N concentrations when compared to wheat monoculture (WW). This effect seems to be less linked with microbial diversity per se than with the *Rhizobium* species present because both microbial analyses found no differences in soil microbial activity, richness or diversity with increasing crop diversity. However, cluster analysis did separate WW from rotation treatments based on richness and diversity scores in combination. The weak effect of crop rotation on microbial diversity and richness is likely due to other abiotic drivers of microbial community structure such as P availability, Na and K excess, and pH, all of which correlated to microbial activity and functional richness in our study. The role that microbial diversity plays in the agroecosystem diversity-function relationship remains complex as revealed by the lack of correlation between functional and genetic diversity scores. However, the relation between crop diversity and functional components including wheat yield and soil N followed a hump-shaped curve, i.e. a rotation with one legume crop was more productive than wheat alone or wheat plus two legumes. Considering the bulk soil N was similar between treatments this suggests that rhizosphere soil N may be higher where *Rhizobium*-colonized medic provides additional N to the wheat crop. This supports the hypotheses of functional redundancy in genetically diverse microbial communities and the selection effect in genetically

homogenous ones. Overall, wheat-legume crop rotations may not rely on microbial diversity per se for the ecosystem services supporting increased yield. Rather the role of specific functional groups in the yield increase of crop rotation systems requires further investigation.

Key Words:

Agroecosystem; ARISA; CLPP; crop rotation; microbial diversity; soil fertility

3.1. Introduction

Conventional agricultural intensification aimed at increased production regardless of negative environmental impacts is not sustainable as it compromises the ecosystem services supporting it (Power, 2010). Conservation agriculture, characterised by minimal soil disturbance (no-till), permanent soil cover, and crop rotation has evolved as a sustainable alternative to conventional agriculture (Hobbs et al., 2008). There is growing recognition in the literature that conservation agriculture practices enhance the delivery of regulating and supporting ecosystem services within agroecosystems (Palm et al., 2014). Crop rotations are one of the under-studied components of conservation agriculture although they have been adopted prior to the Green Revolution for their yield enhancing effects (Bullock et al., 1992). The yield increase associated with crop rotations has been attributed to ecosystem services such as soil fertility, water-use efficiency, maintenance of soil structure, and disruption of pest cycles (Smith et al., 2008). It is widely understood that the delivery of these services are mediated by the microorganisms within the soil as they are responsible for many biogeochemical reactions in soils concerning nutrient cycling and climate mitigation (de Vries et al., 2013). Thus, it is important to understand how crop rotation effects rhizosphere biochemistry as well as the exploitation of alternative soil niches, and how this links to soil microorganisms and agroecosystem function.

Crop rotations alter soil physico-chemical properties and are thus predicted to alter soil biological parameters such as microbial abundance and diversity. In a meta-analysis of 122 studies, McDaniel et al. (2014) found that adding additional crops in rotation to a monoculture increased soil microbial biomass, carbon (C) and N pools. It is, however, unclear, as to whether this increased microbial abundance was associated with increased diversity. It has been posited that aboveground biodiversity is correlated with belowground biodiversity (Hooper et al., 2000). A diversity of root exudates and plant litter build-up from residual crops in rotation is predicted to lead to a greater diversity and abundance of microorganisms (Costa et al., 2006; Kent and Triplett, 2002). Lupwayi et al. (1998) discovered that microbial diversity,

based on community level substrate utilisation (respiration), was significantly higher under a rotation of wheat and clover or field peas than under continuous wheat monoculture. However, other studies have shown that crop rotation has limited or negative effects on microbial diversity. For example, Navarro-Noya et al. (2013) found that crop management (continuous maize versus maize-wheat rotation) had no effect on soil microbial diversity. Yin et al. (2010) found that incorporating soy beans into rotation with wheat decreased richness and Shannon-Weaver diversity indices in conventional tilled plots when compared to continuous wheat. Therefore, the relationship between increasing crop diversity and soil microbial diversity is not clear. Besides this, the relationship between microbial diversity and agroecosystem functioning is also poorly understood (Nannipieri et al., 2003; van der Heijden and Wagg, 2013).

Microorganisms mediate nutrient cycling delivery through the decomposition of organic material, thereby enhancing soil fertility, soil structure and water use efficiency (Kennedy, 1999). The relative diversity and abundance of microbial taxa can enhance the redundancy, resilience and stability of agroecosystems (Giller et al., 1997). High microbial diversity acts as an insurance against ecosystem malfunctioning due to multiple genetic sub-units that can perform the same function and have variable tolerances to stress. However, other studies suggest that increased agroecosystem functioning is influenced by key-stone microbial species (e.g. N-fixers, specific pathogens) and not diversity or abundance per se (Sharma et al., 2011). Crop rotations which include legumes in rotation enhance soil fertility through the symbiotic relationship with *Rhizobium* bacteria, which fix atmospheric N into the soil (Hansen, 1994). There are, however, a diversity of free-living bacteria that can also contribute to bulk soil N. Thus it can be expected that bulk soil N responds to changes in total soil microbial diversity and not *Rhizobium* species alone. Within the broader context of diversity-ecosystem function theory, an increase in crop and/or microbial diversity may result in a linear, curvilinear or poorly curvilinear relationship with the agro-ecosystem function (Smith et al., 2008; Vitousek and Hooper, 1994). Alternatively, it has been proposed that the well-known hump-shaped relation between biodiversity and function in higher plants may as well be true for soil microbial biodiversity (Anderson, 2003; Nannipieri et al., 2003). It could thus be expected that with increased microbial diversity there is increased soil fertility until a certain point is reached, thereafter the inverse is true.

Recent advances in molecular and biochemical analytical tools to assess soil microbial communities have improved on previous culture-based techniques in assessing and comparing genetic and functional microbial community structures (Kent and Triplett, 2002; Sharma et al., 2011). These include community-level physiological profiling (CLPP) and automated rRNA intergenic spacer analysis (ARISA). CLPP gives an indication of the

functional diversity within a soil sample by measuring the potential utilisation of 31 different naturally-occurring carbon sources (Garland, 1997). ARISA gives an indication of the genetic diversity within a soil by measuring the relative abundance of rRNA intergenic spacer amplicons of varying lengths representing separate genetic sub-units or operational taxonomic units (OUT's) (Ranjard et al., 2001). To gain clearer insight into microbial processes in soil ecosystems and assess the effect of agricultural practices it is essential to study functional and genetic diversity in combination with one another (Kent and Triplett, 2002). To our knowledge, no study has used both forms of microbial analysis to look at the effects of crop rotation on soil microbial populations and their function.

This study investigates the links between crop rotation, microbial biodiversity and agroecosystem function at a long-term rotation trial in the Swartland Local Municipality of South Africa. To do this, (1) CLPP and ARISA were used to assess the impact of wheat-legume crop rotation on soil microbial activity, richness and diversity, and (2) the associated effect of the rotations on soil N content, general soil fertility and wheat plant yield was correlated with microbial diversity and function.

3.2. Materials and methods

3.2.1. Experimental site

The study was conducted during the winter growing season from August to November 2013. Sampling was performed within a 19-year, long-term crop rotation trial initiated in 1996 at Langgewens Experimental Farm near Mooresburg in the Western Cape of South Africa (18.700 E, 33.283 S). This important wheat-producing region experiences a Mediterranean climate with wet, cool winters and hot, dry summers. Long-term ($n = 40$ years) mean daily minimum and maximum temperatures range between 10.7°C and 22.3°C between August and November (P Lombard, Western Cape Department of Agriculture, pers. comm.). Long-term average annual precipitation is 394.8 mm per annum, with 473 mm falling during 2014. Soils are of the Mispah and Glenrosa soil forms, consisting of shallow (200 to 400 mm) sandy loam with a 45% stone content in the A-horizon. A high susceptibility to water-logging was reason to “ridge-and-furrow” the trial site prior to the start of the trial.

3.2.2. Experimental layout

The long-term trial was set up as an unbalanced randomised block design with a split-plot arrangement. Five two-year rotation treatments, consisting of two 2 ha plots each, were selected for the study. Treatments selected for this study included two-year crop rotations of continuous wheat (WW) (hereafter referred to as 'monoculture') and a two combinations of

wheat/legume-pasture rotation systems (hereafter referred to as 'rotation'). The wheat cultivar used was *Triticum aestivum* cv. SST 027 and the annual legumes included medic (*Medicago trunculata* cv. Parabinga and Sephi) and clover (*Trifolium repens* cv. Balansa and Roos). The rotations consisted of wheat followed by medic (Wm) and wheat followed by a mixed pasture of medic and clover (Wmc). Every year since 1996, two plots for each rotation were planted to an annual legume and two were planted to wheat, thus providing an additional two treatments of medic followed by wheat (mW) and medic/clover mix followed by wheat (mcW). The inclusion of both alternate rotation years within the treatments accounts for climate variability. Based on the assumption of soil homogeneity within sampling plots, each plot was divided into three sub-plots, creating six replicates per treatment (n=6). In addition to the treatments, a protected area of Renosterveld located near the experimental farm was used as a reference (Ref) site.

3.2.3. Crop management

Principles derived from conservation farming practices were used in the planting, protection and harvesting of the crop. Wheat planting is achieved with a no-till planter, which disturbs approximately 20% of the soil surface. Pasture crops were planted in 1996 and each subsequent pasture production year, the legume plants regenerated from soil-stored seed banks or supplemented with plantings if needed. Thus, soil disturbance occurred every year in the monoculture plots and every second year in the rotation plots. Soil macro- and trace elements were maintained at recommended levels for each crop according to Anon (1990). Weeds were controlled post-germination with broad spectrum herbicides including 750 g.kg⁻¹ Triasulfuron and 360 g.L⁻¹ Glyphosate before planting. Prior to 2001, all residue wheat straw was removed from the plots after harvesting. Post 2001, residue crop was retained on the plots and sheep were allowed to graze the residue in the rotation plots only. In 2008, the monoculture plots were burned post-harvest in an attempt to reduce the herbicide resistant ryegrass infestation. Sheep are stocked on the pasture rotation a few weeks after planting and remain there for the winter at a stocking density of four ewes per hectare.

3.2.4. Sampling procedure

Wheat plants were sampled in the monoculture plots and wheat-phase of the rotation plots (WW, Wm and Wmc). Historical yield data for the experimental plots was obtained from Langgewens Experimental Farm. This was measured in tonnes per hectare each year after harvest with a combine harvester at the point when the water content of wheat grain was less than 13%. In addition to the field-based harvest, six plants were randomly selected and tagged per sub-plot (i.e. 18 per treatment) and stem length was measured on three occasions at the beginning (August), middle (September) and end (October) of the growing season. Tagged

plants were removed at the shoot-root base, and weighed for wet mass after the final measurement of stem length. The samples were subsequently placed in brown paper bags and dried at 70°C in an oven for five days. The shoots were then re-weighed for dry mass and analysed for macro- and trace elements using inductively coupled plasma optical emission (ICP-OES, ICP 6000 series, Thermo Eioctron Corp.) after dry-ashing.

Soils were sampled before harvest at the end of October using a rocky soil auger (40 mm in width) to a depth of 70 mm and 150 mm for microbial and nutrient analyses, respectively. After removing surface organic matter, six samples were collected per sub-plot around the tagged plants from within wheat rows and pooled together for the microbial analysis. Each soil sample comprised three soil cores which were subsequently mixed. Soil was immediately sieved through a 2-mm sieve, collected onto ice and stored at 4°C for subsequent DNA extraction and CLPP profiling. For soil nutrient analysis soil samples were air-dried for three days and subsequently analysed for macro- and trace elements using ICP-MS as previously described where NH₄⁺-nitrogen was analysed using routine Kjeldahl digestion.

3.2.5. Community-level physiological profile (CLPP)

Substrate utilisation patterns of culturable soil microbial (bacterial) communities, as a measure of functional diversity, were assessed using Biolog-EcoPlate™ (Biolog Inc., Hayward, CA, USA) according to a procedure adapted from Garland and Mills (1991). A pre-incubation was performed to allow microbial utilisation of residual soluble organic carbon present in the soil. To do this, 5 g dry weight soil samples were moistened to 40% water holding capacity and incubated for 6 days at 25°C. Samples were covered in Parafilm® to allow for CO₂ and O₂, but not H₂O exchange. After incubation, samples were shaken with 40 mL of 0.8% NaCl buffer solution for 30 minutes on an orbital shaker. After sediment had settled, 5 mL of supernatant was removed and centrifuged softly at 300 g to pelletize remaining sediment. Thereafter, 125 µL aliquots of the supernatant were used to inoculate each well of the Biolog-EcoPlates™. Six EcoPlates were used per treatment (one per replicate), which contained internal triplicates of 31 different carbon sources suitable for soil microbes (Choi and Dobbs, 1999). Plates were stored on an orbital shaker at 25°C. The utilisation of the carbon sources (indicated by a reduction of the tetrazolium dye) was then recorded on a Bio-Rad Micro Plate Reader at 590 nm at 24, 48, 72 and 96 h after inoculation. Absorbance values were corrected by subtracting the control well from the actual reading. Any negative results after correction were recorded as zero.

Total microbial activity is expressed as average well colour development (AWCD), calculated as $AWCD = \frac{\sum OD_i}{31}$, where OD_i is the ratio of the corrected optical density value of each well. Microbial diversity was determined using measures of richness and evenness by comparing

plates that most approximate an AWCD of 0.75 (Garland, 1997). The threshold for a positive test was determined as any value, after background correction, exceeding 0.25. Richness (S) was determined as the count of positive testing absorbance values. Evenness was determined using the Shannon-Weaver index (H) as $H = -\sum p_i(\ln p_i)$, (Shannon and Weaver, 1969) where p_i is the ratio of the corrected absorbance value of each well to the sum of the absorbance value of all wells at a wavelength of 590 nm. Comparisons of the pattern of carbon source utilisation between treatments were achieved using Principle Components Analysis (PCA).

3.2.6. Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Total microbial DNA was extracted from soils within a week of field sampling. The bacterial ARISA, as a measure of genetic diversity, involved the extraction of total community DNA from soil samples, PCR amplification using fluorescence-tagged oligonucleotide primers targeting the intergenic spacer region transcribed from between the small (16S) and large (23S) subunit of the rRNA, laser detection of fluorescent DNA fragments, and analysis of banding patterns. Total DNA was extracted from 0,35 g of moist soil using ZR Soil Microbe DNA kit (Zymo Research, California, USA) following the manufacturer's instructions and stored at -18°C until further analysis.

The PCR reactions were carried out using Bacterial specific primers, ITSReub and FAM (carboxy-fluorescein) labelled ITSF (Cardinale et al., 2004). For each treatment, consisting of six samples, the reaction mixture contained 40 µL of 2X KapaTaq Readymix (KapaBiosystems, South Africa) master mix, 24 µL of ultrapure water (Milli-Q®), and 4 µL of each primer. Amplification was performed on a GeneAmp® PCR System 9700 machine using the following cycling parameters: 5 min at 94°C, 30 cycles of 30 s at 94°C, 45 s at 56°C, 1 min 10s at 72°C, and a final 5 min at 72°C. This was repeated three times for each sample set so as to create triplicate results which were then pooled into one Eppendorf sample tube. PCR samples were separated on a 1% agarose gel, stained with ethidium bromide and visualized using ultraviolet light. The amplicons from bacterial specific PCR were run on an ABI 3010xl Genetic analyser to obtain an electropherogram of the different fragment lengths and fluorescent intensities. ARISA samples were run with ROX 1.1 size standard which varied from 20 to 900 bp (Slabbert et al., 2010). ARISA data was analysed using Genemapper 4.1 software, which converted fluorescence data to an electropherogram representing fragments of different sizes. Only fragment sizes larger than 0.5 % of the total fluorescence, ranging from 120 to 1000 base pairs in length was considered for analysis. A bin size of 3 bp for fragments below 700 bp and 5 bp for fragments above 700 bp was employed to minimise the inaccuracies

in the ARISA profiles (Ranjard and Nazaret, 2000; Slabbert et al., 2010). Shannon-Weaver and richness scores were calculated using data OTU's as determined from the ARISA data.

3.2.7. Statistical analysis

Differences in treatments based on measured variables were calculated using analysis of variance (ANOVA, cut-off for significant $p < 0.05$) with Statistica v.12 (StatSoft, Dell Inc. USA). There was no significant difference between blocks for any of the tests performed, thus blocks were pooled per treatment and not included as a categorical factor. A cluster analysis using Euclidean distances was performed using diversity and richness scores as factor variables for the CLPP and ARISA data separately to assess the grouping relationships between treatments. A PCA was used to analyse the CLPP data after substrates were divided into six groups and the average absorbance per category was calculated (Zak et al., 1994). Meaningful loading variables (> 0.5) were considered as significant in the interpretation of principle components (Manly, 1994). Regression analyses were performed on richness and diversity scores, as well as CLPP and ARISA results. Correlation matrices were constructed using microbial diversity, richness and activity scores, plant and soil nutrient levels, and yield data.

3.3. Results

3.3.1. Yield

Wheat plant stem length was higher in rotation treatments compared to WW ($p < 0.0001$), however historical wheat yield did not differ between treatments ($p = 0.257$, Figure 3.1). Biomass data from tagged plants showed that there was no difference in individual plant leaf ($p = 0.935$) or grain ($p = 0.202$) mass between treatments.

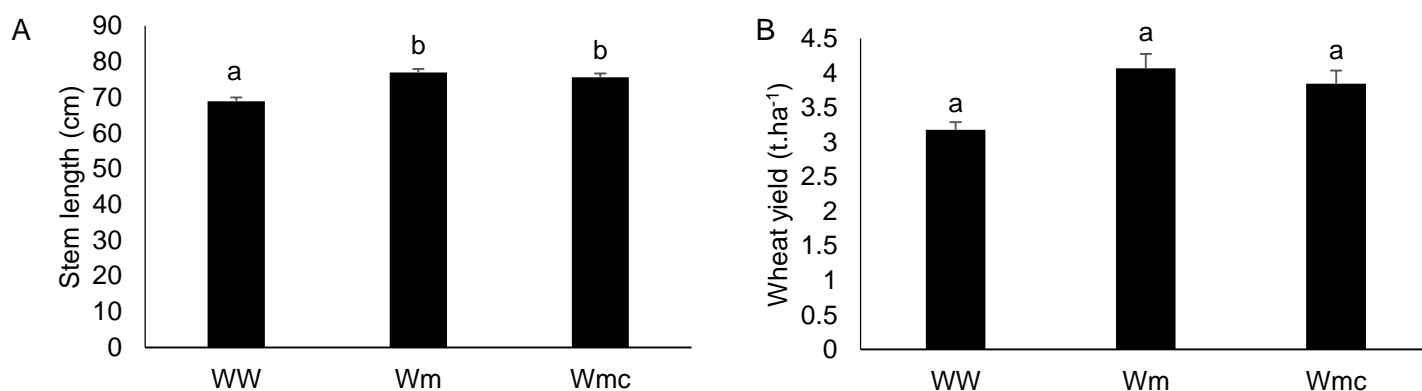


Figure 3.1: Accumulative wheat stem length measured at three time points during the 2013 growing season (A), and total wheat grain yield for the past 10 years (B). Whiskers indicate standard error. Different letters above the bars indicate least squared means of treatments from a repeated measures ANOVA at the $P < 0.05$ level. Abbreviations: WW, wheat/wheat; Wm, wheat/medic; Wmc, wheat/medic&clover

3.3.2. Functional diversity (CLPP)

Cluster analysis based on microbial activity, richness and diversity scores revealed a separation in microbial community function in response to rotation treatments (Figure 3.2A). The WW monoculture was clearly separated from rotation treatments and the Ref site at the highest Euclidean distance. The crop rotations as a whole were more similar to the natural (Ref) site than to the monoculture (WW) although this was at a relatively high level (2nd order). The mW and Wmc treatments containing legumes were most similar with the lowest Euclidean distances.

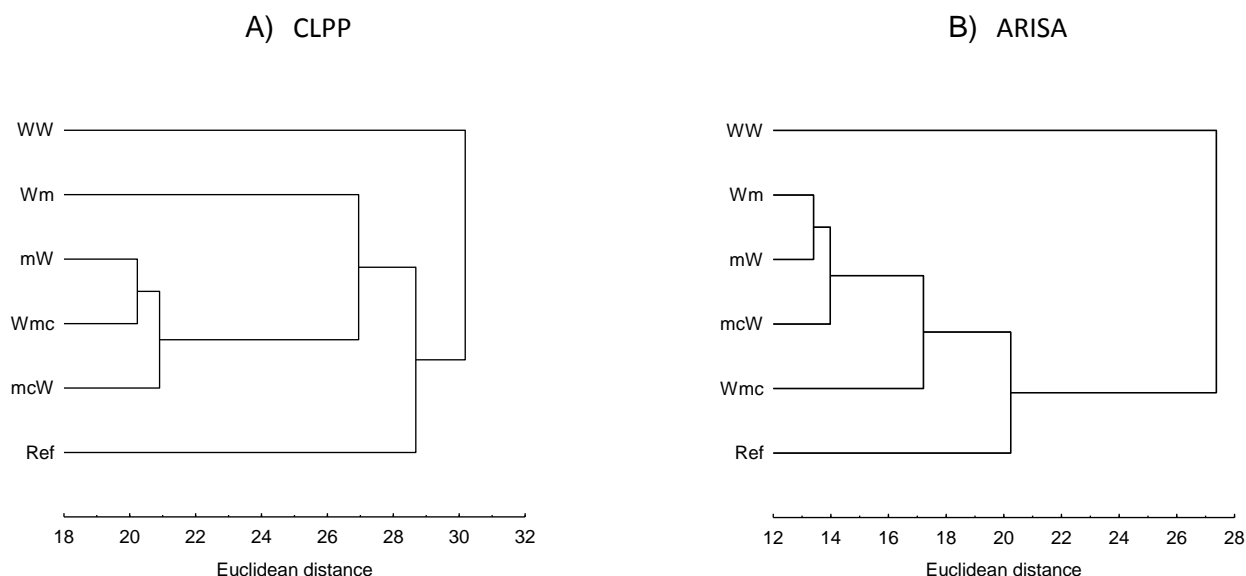


Figure 3.2: Cluster analysis of community-level physiological profiles (CLPP) and automated rRNA intergenic spacer analysis (ARISA) of soils. The dendrogram was based on Euclidean distances calculated from richness (S) and Shannon-Weaver diversity (H) scores. Monte Carlo permutation testing on all six field replications was used to determine significant branching in the dendrogram. Abbreviations: WW, wheat/wheat; Wm, wheat/medic; mW, medic/wheat; Wmc, wheat/medic&clover; mcW, medic&clover/wheat; Ref, reference.

The overall microbial activity, as measured by utilisation of C substrates (AWCD), was not significantly different between rotation treatments when the Ref site was excluded from the model ($p = 0.142$, Figure 3.3). However, the highest average level of microbial activity occurred in the Wmc rotation (optical density of 0.744), whereas WW monoculture produced the lowest value for microbial activity (optical density of 0.581) amongst the treatments. When Ref was

added to the model, a post-hoc Tukey HSD test revealed significant differences between Ref and mW ($p = 0.036$) and Wmc ($p = 0.002$). The AWCD (microbial activity) generally followed the same pattern between treatments as it increased with incubation time (Figure 3.3).

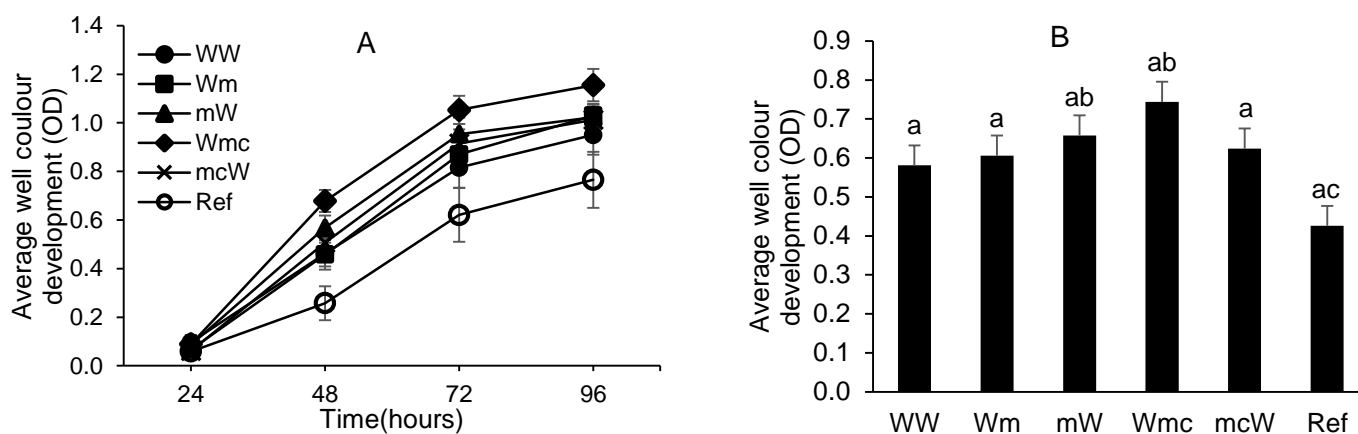


Figure 3.3: Average well colour development over a 96 hour time course, measured as optical density (OD), from community-level physiological profiles obtained by Biolog-EcoPlate™ inoculation of treatment soil samples with data showing averages \pm standard errors (A); and least squared means of treatments from a repeated measures ANOVA where a significant difference is indicated by differing letters above bars at the $P < 0.05$ level (B). Abbreviations: WW, wheat/wheat; Wm, wheat/medic; mW, medic/wheat; Wmc, wheat/medic&clover; mcW, medic&clover/wheat; Ref, reference.

Increasing crop diversity through wheat-legume rotations did not significantly affect the microbial community functional richness ($p = 0.5127$) or diversity ($p = 0.263$) as measured by CLPP. Rotations of wheat with medic tended to have the highest average richness ($S = 69$) and diversity ($H = 4.21$) scores for the mW and Wm treatments, respectively. Rotation treatments sampled in the legume phase of the rotation (mW, mcW) also tended to have higher average richness scores than those in the wheat phase (Wm, Wmc), while the same was not the case for diversity scores. The Ref consistently produced the lowest average microbial activity, richness and diversity scores with the highest levels of variability between replicates.

The PCA showed no distinct separation of treatments, nor any specific correlations between treatments and types of substrate utilisation (loading variables comprising of six groups of carbon substrates, Figure 3.4). The proportion of variation explained by PC1 was 72.9% and the loading variables, comprising of six categories of Biolog-EcoPlate™ C substrates, contributed toward the spread of variables along PC1 and PC2. Microbial substrate utilisation of all substrates was significant (factor co-ordinates $> \pm 0.5$) in separating the treatments on the PC1 axis. On the PC2 axis, amide and miscellaneous substrate metabolising bacteria had the greatest influence on the treatments with factor co-ordinates of -0.418 and 0.537

respectively. The Ref treatment replicates were negatively correlated with the loading variables, whereas Wmc replicates were positively correlated.

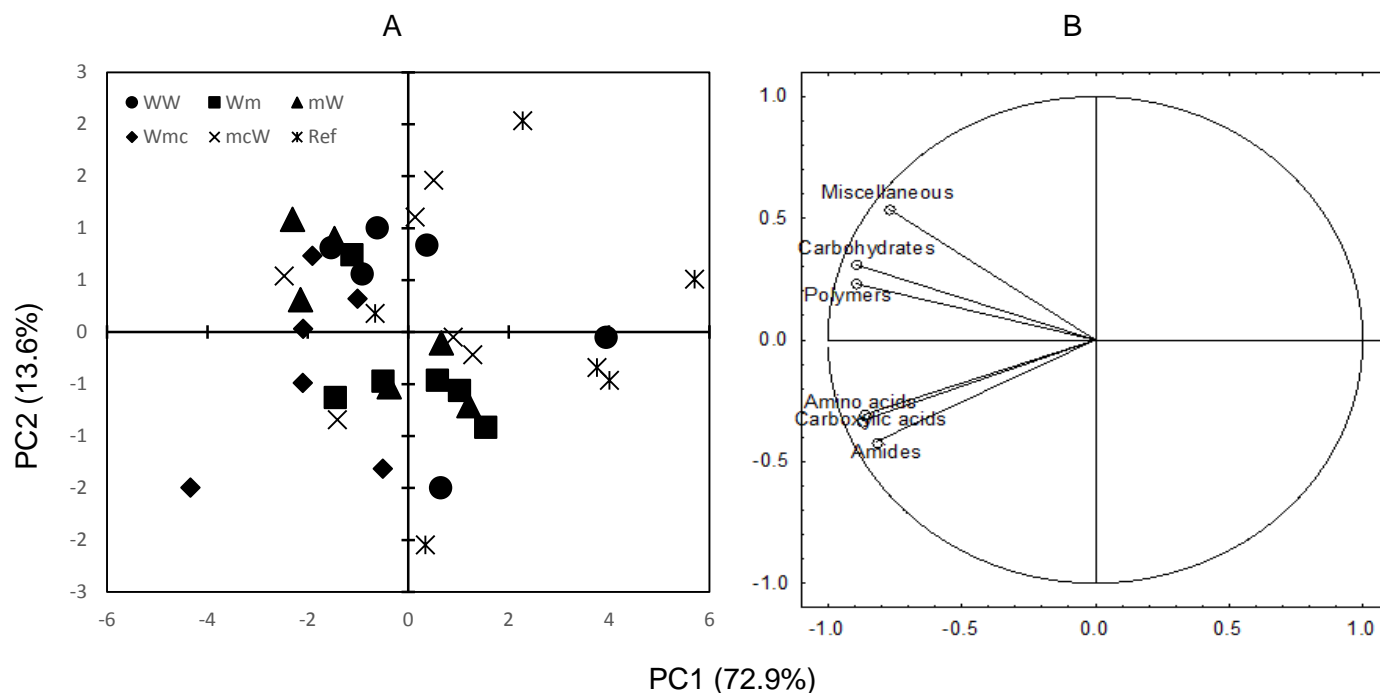


Figure 3.4: Principle Components Analysis of the absorbance data from Biolog-Ecoplate™ community-level physiological profiles inoculated with soils. Abbreviations: WW, wheat/wheat; Wm, wheat/medic; mW, medic/wheat; Wmc, wheat/medic&clover; mcW, medic&clover/wheat. Loading variables based on rotation treatments include six categories of carbon substrates. Treatment scatterplot (A) and loading variables (B) on PC1 and PC2 axis.

3.3.3. Genetic diversity (ARISA)

Similarly to CLPP, cluster analysis based on ARISA richness and diversity scores revealed a separation in microbial community structure in response to rotation treatments (Figure 3.2B). The WW monoculture was clearly separated from other rotation treatments and the Ref site at the highest Euclidean distance, although Ref was still differentiated from the rotation treatments at a relatively high Euclidean distance. Also similar to the CLPP data, Wm and mW treatments were most similar with the lowest Euclidean distances.

As indicated by CLLP, ARISA indicated that there was no significant difference between rotation treatments in microbial genetic richness ($p = 0.563$) and diversity ($p = 0.454$). This result did not change when the Ref was included in the model. The WW monoculture tended to produce the lowest average genetic richness ($S = 41.5$) and diversity ($H = 3.24$) scores, while the Ref site produced the highest ($S = 46.3$, $H = 3.57$). Rotation treatments in the legume phase of the rotation (mW, mcW) tended to produce lower average richness and diversity scores than those in the wheat phase (Wm, Wmc).

3.3.4. Methodological comparison

The relationship between richness and diversity scores showed a significant positive correlation for both CLPP ($p < 0.0001$, $R = 0.493$) and ARISA ($p < 0.0001$, $R = 0.851$) data, representing functional and genetic diversity respectively. Regression analysis on the CLPP and ARISA data revealed no correlation for richness scores ($p = 0.11$, $R = 0.078$) and diversity scores ($p = 0.528$, $R = 0.012$).

3.3.5. Nutrient cycling

Soil N and C did not differ between treatments ($p = 0.469$, $p = 0.167$, Table 3.1). As expected for a soil of the Fynbos Biome, soil phosphorous levels were lower at the reference site compared to the agricultural sites ($p < 0.001$) where the latter had been regularly fertilized. Post hoc Tukey HSD tests revealed that Wmc treatment produced significantly higher wheat plant N ($p < 0.001$), P ($p < 0.001$) and Mg ($p < 0.001$) levels and lower grain N ($p < 0.01$) and P ($p < 0.001$) levels relative to WW (Table 3.1).

Table 3.1: Soil, wheat plant and grain average nutrient levels for rotation treatments at Langgewens Experimental Farm. Abbreviations: WW, wheat/wheat; Wm, wheat/medic; mW, medic/wheat; Wmc, wheat/medic&clover; mcW, medic&clover/wheat. Numbers are averages \pm standard error, with significant differences indicated by differing letters at the $P < 0.05$ level.

Rotation	NH ₄ ⁺ -N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (cmol kg ⁻¹)	Na (mg kg ⁻¹)	Ca (cmol kg ⁻¹)	pH	C (%)	S (%)
Soil									
WW	0.11 \pm 0.01 a	80.33 \pm 3.89 a	143.97 \pm 16.96 a	0.95 \pm 0.16 a	18.5 \pm 1.41 a	5.84 \pm 0.75 a	6 \pm 0.2 ab	1.41 \pm 0.11 a	-
Wm	0.13 \pm 0.01 a	92.17 \pm 3.31 a	110.67 \pm 11.44 ab	1.11 \pm 0.07 a	22.83 \pm 3.17 a	9.22 \pm 1.93 ab	6.43 \pm 0.12 a	1.47 \pm 0.1 a	-
mW	0.11 \pm 0.01 a	76.33 \pm 8.85 a	119.33 \pm 10.98 ab	1.09 \pm 0.16 a	22 \pm 1.13 a	5.59 \pm 0.56 a	6.18 \pm 0.11 a	1.26 \pm 0.14 a	-
Wmc	0.13 \pm 0.01 a	92.33 \pm 8.22 a	95.5 \pm 17.64 ab	1.25 \pm 0.32 a	17.33 \pm 0.92 a	6.17 \pm 0.92 a	6.17 \pm 0.2 a	1.54 \pm 0.17 a	-
mcW	0.11 \pm 0.01 a	94.83 \pm 11.8 a	155.83 \pm 33.49 a	0.64 \pm 0.07 a	23.17 \pm 0.83 a	4.71 \pm 0.24 ac	5.88 \pm 0.14 ab	1.28 \pm 0.11 a	-
Ref	0.12 \pm 0.01 a	29.17 \pm 3.36 b	217 \pm 28.3 ac	1.43 \pm 0.23 a	35.5 \pm 4.71 b	3.24 \pm 0.42 ac	5.38 \pm 0.18 ac	1.73 \pm 0.16 a	-
Plant									
WW	0.43 \pm 0.02 a	0.06 a	1.73 \pm 0.06 a	0.07 a	240.38 \pm 14.61 a	0.21 \pm 0.01 a	-	-	0.1 \pm 0.01 a
Wm	0.58 \pm 0.04 a	0.07 a	2.17 \pm 0.15 b	0.09 \pm 0.01 b	376.19 \pm 21.16 b	0.27 \pm 0.01 b	-	-	0.14 \pm 0.01 b
Wmc	1.18 \pm 0.22 b	0.2 \pm 0.05 b	1.34 \pm 0.35 c	0.1 \pm 0.01 c	242.14 \pm 37.03 a	0.18 \pm 0.03 a	-	-	0.1 \pm 0.01 a
Grain									
WW	1.47 \pm 0.07 a	0.3 \pm 0.01 a	0.66 \pm 0.04 a	0.11 a	155.18 \pm 8.57 a	0.08 \pm 0.01 a	-	-	0.1 a
Wm	1.66 \pm 0.05 b	0.34 \pm 0.01 a	0.71 \pm 0.05 a	0.12 b	145.55 \pm 10.25 a	0.09 a	-	-	0.1 \pm 0.01 b
Wmc	1.19 \pm 0.22 c	0.21 \pm 0.06 b	1.19 \pm 0.21 b	0.1 \pm 0.01 a	265.56 \pm 67.06 b	0.18 \pm 0.04 a	-	-	0.1 c

3.3.6. Agroecosystem relationships

Yield was positively correlated to crop diversity and genetic microbial diversity (Figure 3.5). However, yield was negatively influenced by the functional diversity of microbes with a significant negative correlation to richness scores at $p < 0.005$ ($r = -0.89$). Increased soil N positively correlated to yield and grain N, however, neither genetic nor functional diversity had any influence on soil N or, correspondingly on grain N (Figure 3.5). Microbial activity was negatively correlated to other soil nutrients including Na ($r = -0.524$) and K ($r = -0.53$), yet positively correlated to P ($r = 0.433$). Furthermore functional microbial diversity was negatively correlated to Na ($p = -0.499$).

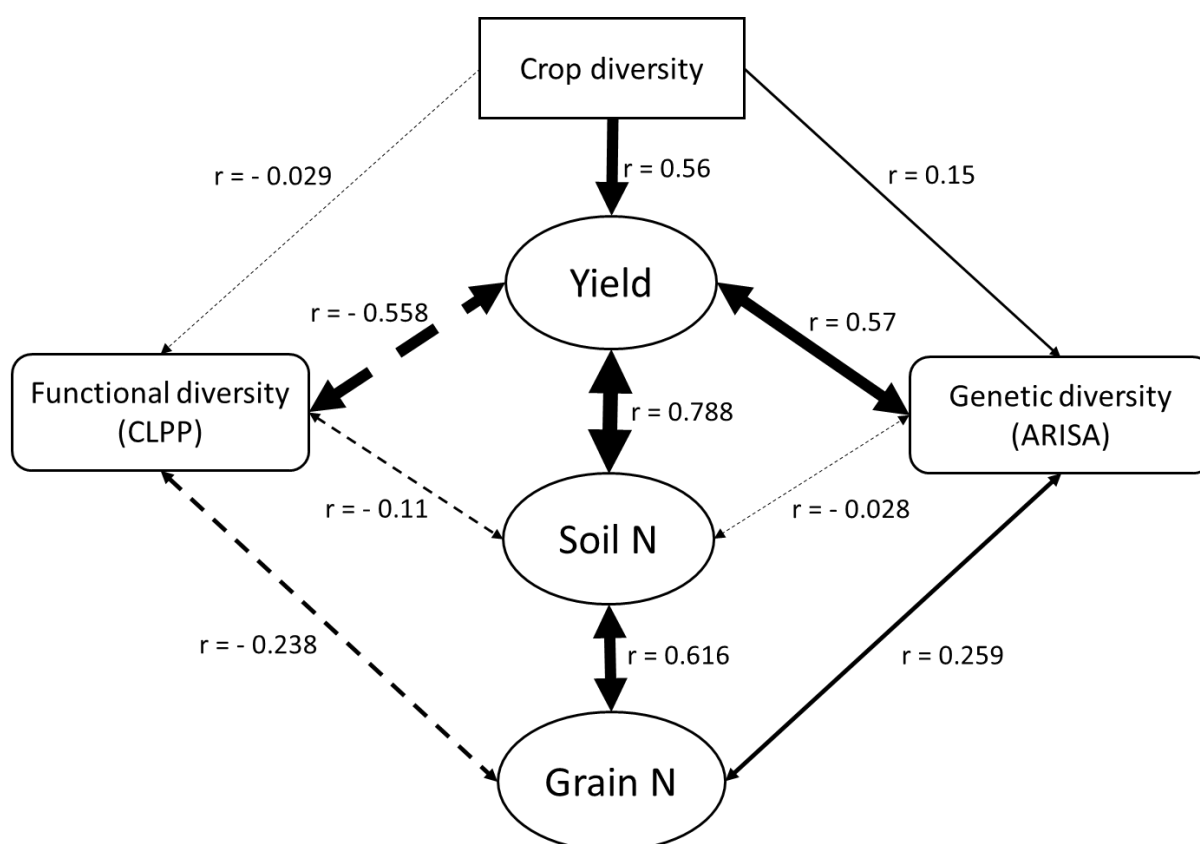


Figure 3.5: Diagram indicating possible relationships as well as correlation coefficients for various agroecosystem diversity and function components across all treatments in this study. Arrow weight indicates strength of correlation with dashes representing negative relationships.

3.4. Discussion

The objective of this study was to investigate the links between crop rotation, microbial biodiversity and agroecosystem function in terms of soil fertility and yield. Different wheat-

legume crop rotation treatments and a wheat monoculture at a long-term trial in the Western Cape of South Africa were assessed for microbial functional and genetic diversity using CLPP and ARISA techniques respectively. Soil and plant nutrient levels, and wheat yield were also sampled as measures of agroecosystem function.

3.4.1. Yield

Wheat-legume rotations resulted in higher plant production (stem length) than wheat monoculture. Although rotations did not produce significantly higher grain yields, at the farm level, historically higher yields and economic gains per hectare from wheat-legume rotations has encouraged the adoption of crop rotation within the region (Hardy and Strauss, 2011). Yield was positively correlated to soil N and grain N which confirms the positive plant physiological response to soil nutrients. Further, this confirms the broadly-recognised beneficial yield effect of crop rotations (Bullock, 1992; Smith et al., 2008).

3.4.2. Soil microbial diversity

The role that soil microbial diversity played in mediating the increase in plant production remains complicated based on our results. The cluster analysis did separate wheat monoculture from the rotation treatments for both CLPP and ARISA when richness and diversity scores were combined (Figure 3.2A). However, the independent ANOVAs of richness and diversity scores revealed that rotations did not significantly alter the functional (CLPP) or genetic (ARISA) microbial diversity of the soil.

The weak and conflicting effects of crop diversity on functional microbial diversity are reflected in the literature as reviewed in Chapter 1 of this thesis. Previous studies employing CLPP as a method have shown positive (Lupwayi et al., 1998; Murphy et al., 2011) or non-significant (Marais et al., 2012; Navarro-noya et al., 2013) effects of crop rotations on functional microbial diversity. Although fungal diversity was not measured in this study (EcoPlate™ tetrazolium dye is not metabolised by fungi), fungal diversity in soils may also be unaffected by crop rotation (Stefanowicz, 2006; Mathimaran et al., 2007). In these studies, other abiotic factors such as soil moisture and pH were found to be driving factors. Given that the Ref site had lower levels of microbial activity yet similar levels of diversity in comparison to the diversified cropping systems, it is possible that latent microbial biodiversity is unaffected by anthropogenic impacts, yet the activity and functioning of that biodiversity is.

Conflicting evidence is also found in studies employing molecular-based methods, such as ARISA, measuring genetic diversity in crop rotations. Guong et al. (2012) and Yao et al. (2006) found lower microbial diversity in soils under monoculture compared to those under rotation. However, other studies on genetic diversity show a lower microbial diversity in soils under

grain-legume rotations than under grain monocultures (Mathimaran et al., 2007; van Elsas et al., 2002; Yin et al., 2010). There are also studies revealing contrasting effects of grain-legume rotations on microbial diversity depending on the study trial location and rotation length (Alvey et al., 2003; Azziz et al., 2012).

3.4.3. Methodological comparison

The results of the methodological comparison revealed no significant relationship between functional diversity (measured with CLPP) and genetic diversity (measured by ARISA). The results are counter to other studies comparing CLPP and phospholipid fatty acid analysis (PLFA, as a measure of species composition) which found that the degrading capacity of a community (CLPP) reflects the species composition (PLFA) (Söderberg et al., 2004). Functional diversity is a function of both genetic variability and phenotypic expression influenced by the ecological interactions between organisms and the environment (Zak et al., 1994). Thus, one possible explanation for the results in this study is that the ecological relationships between microbial species complicates the effect of genetic diversity on functional diversity. For example, at low levels of genetic diversity there can be higher-than-expected levels of functional diversity because of functional redundancy where there are multiple taxonomic sub-units that can perform the same function (Nannipieri et al., 2003). Thus although WW had lower genetic diversity, this may not have impaired the associated functional diversity of the soil microbial communities.

The weak relationship between CLPP and ARISA may also be due to problems intrinsic to the methodology. CLPP only measures the activity of a part of the total microbial community, probably mainly fast-growing bacteria, yeast and fungi, and may misrepresent the diversity of actual carbon sources in the soil (Stefanowicz, 2006). ARISA is vulnerable to underrepresenting taxonomic diversity due to the overlapping size classes among unrelated populations (Ranjard et al., 2001). Furthermore, microbial community composition may differ with crop rotations, yet this would not be identified with CLPP or ARISA unless further detailed genetic analysis is performed. Nevertheless, these methods used in combination can give important reliable and repeatable insights into soil microbial diversity (Torsvik & Øvreås, 2002).

3.4.4. Nutrient cycling

The conflicting evidence for a rotation effect on functional or genetic microbial diversity across the literature and within our study suggests that there are multiple abiotic and biotic factors driving diversity which differ over time and space. One mechanistic hypothesis for an increase in microbial diversity suggests that crop rotations increase soil organic matter quantity and quality, which in turn stimulates microbial abundance and functional diversity. Soil nutrient analyses revealed no differences in N and C across treatments, which could explain the

similarity in microbial diversity. McDaniel et al. (2014) found in a meta-analysis that studies including cover crops in rotation resulted in increased bulk soil C which had an associated increase in microbial biomass. Other studies have found functional links between the changes in quality and quantity of soil organic matter and microbial composition (Bird et al., 2011; Cusack et al., 2011). Thus, if our study found differences in soil C, perhaps this may have increased microbial biomass and possibly diversity. In terms of soil organic matter quality, our study found higher N and lower P levels in wheat plants (residue) under the Wmc treatment when compared to WW. It could be expected that if this plant material becomes residue that is incorporated into the soil, the quality of soil organic matter and subsequently the microbial diversity could change significantly, yet this was not the case.

Microbial activity was correlated to soil nutrients other than N and C, where significantly lower P levels in the Ref site correlated to lower microbial activity. Significantly higher Na and K levels in the Ref site also correlated to lower microbial activity and functional diversity. In addition, although not correlated with microbial diversity, pH was significantly lower in Ref site compared to WW and mcW. Thus P availability, Na and K excess, and pH may be drivers of microbial activity and possibly diversity. This supports a broad-scale study on bacterial abundance and diversity patterns along precipitation gradients in Mediterranean, semi-arid and arid sites, which showed that bacterial abundance in soil is correlated with water availability, but the richness and diversity of communities were influenced by nutrient availability and pH (Bachar et al., 2010).

3.4.5. Agroecosystem relationships

It is difficult to determine whether soil physico-chemical factors drive microbial diversity or whether the inverse is true. One of the challenges within microbiology is to understand the link between diversity and function (Torsvik & Ovreas, 2002). Within agroecosystems, crop diversity and microbial diversity may be linked with functional traits such as soil fertility and yield. Broader ecological theories suggest that agroecosystem function may be related to diversity through linear, curvilinear, poorly curvilinear (Smith et al., 2008; Vitousek and Hooper, 1994), or hump-shaped relationships (Anderson, 2003; Nannipieri et al., 2003). In terms of soil N fertility, our study partially aligned with this theory and partially did not: the presence of one additional legume in the crop diversity did correlated with increased plant N, while there was no link between this increased plant N concentration and the microbial diversity. Underlying this is the fact that there was no correlation between crop diversity and microbial diversity. In terms of crop diversity, and crop function in terms of yield and N concentration, our results seemed to follow a hump-shaped or poorly curvilinear curve with rotations of wheat with legumes resulting in higher yields than wheat monoculture (Figure 3.1). This aligns somewhat

with a study by Smith et al. (2008) which shows that over three years, corn yield increased linearly with the number of crops in rotation. It also confirms the recognised positive relationship between crop yields and crop rotation (Bullock et al., 1992).

The extent to which microbial diversity mediated this relationship was not clear as no significant correlation between microbial diversity (functional or genetic) and wheat yield was found (Figure 3.5). However, CLPP richness scores were negatively correlated with yield which contradicts the broader diversity-function theories mentioned above. This suggests that lower functional richness of soil microbes may result in increased agroecosystem function. This provides support for the selection effect hypothesis where ecosystem function is a product of specific productive species, as opposed to the complementarity effect involving the facilitative interaction of a diversity of species (Hooper et al., 2000). Wheat-legume rotations may have lower functional richness, yet the presence of N₂-fixing symbiotic bacteria (*Rhizobia* spp.) results in higher than expected agroecosystem function. Considering the bulk soil N was similar between treatments, yet the wheat plant N was higher in Wmc, it is possible that soil rhizosphere N is patchy, with *Rhizobium*-colonized medic providing additional N to the wheat crop.

3.5. Conclusion

Increasing crop diversity through rotating wheat with legumes positively impacted cash-crop production. Although crop diversity can also impact microbial communities due to changes in agroecosystem properties such as water-use-efficiency and C substrate quality and quantity, this study revealed no significant impact of the diversity of wheat-legume crop rotations on functional and genetic microbial diversity. However, when richness and diversity scores were considered in combination, cluster analysis produced a separation between wheat monoculture and rotations. These results together seem to reflect the conflicting evidence that is also found in the literature on the effect of crop rotation on microbial diversity, suggesting that there are multiple abiotic and biotic factors mediating this effect which differ over time and space. Within our study, likely drivers included P availability, Na and K excess, and pH due to significant correlations with microbial activity and functional richness. Another possible driver is soil organic matter quantity and quality, yet no differences in soil C and N levels with increasing crop diversity were found, which may explain the similarity in microbial diversity across treatments. Further research on crop rotations that significantly alter soil organic matter content may find differences in microbial diversity. Components of agroecosystem function (soil N and yield) did not correlate linearly with components of agroecosystem diversity (crop and microbial diversity). Soil N was not correlated to crop or microbial diversity. Yet the

relationship between crop yield and crop diversity produced a hump-shaped curve, thus aligning with broader ecological theories and suggesting that rotations with one legume crop such as medic are most productive. The extent to which microbial diversity mediated this relationship remains complex as revealed by the lack of significant correlation between CLPP and ARISA scores. This supports the hypotheses of functional redundancy in genetically diverse microbial communities and the selection effect in genetically homogenous communities, where the increased wheat plant N was likely mediated by local-scale increased soil N availability due to N₂-fixing *Rhizobia* in the legumes.

Overall, wheat-legume crop rotations may not rely on microbial diversity per se for the ecosystem services supporting increased yield. Rather the role of specific functional groups in the yield increase of crop rotation systems requires further investigation.

CHAPTER 4: GENERAL CONCLUSION AND FUTURE RESEARCH

The sustainability of our earth's ecosystems appears to be intrinsically linked to the immense biodiversity and its associated variety of functional ecosystem services. Within agroecosystems, post-green revolution conventional agriculture practices do not mimic natural ecosystems in terms of their levels of biodiversity. The simplification of cropping systems through monocultures and the intensification of chemical inputs poses a threat to the sustainability of ecosystem services flowing to and from agroecosystems. It is proposed that adoption of sustainable practices such as conservation agriculture, which promote diversification through crop rotations, may sustain the ecosystem services supporting agroecosystem function particularly in terms of cash crop yield and soil nutrient fertility. It is also proposed that belowground biodiversity has a role to play in mediating the effect of crop rotations on agroecosystem function. This thesis aimed at investigating (1) the relationship between above and belowground diversity in crop rotation systems, and (2) the relationship thereof to agroecosystem function in terms of soil fertility and crop yield.

Results from Chapter 1, revealing trends at a global level, generally agreed with results from Chapter 2, revealing trends within the local context in the Swartland of South Africa. Although the meta-analysis revealed an overall positive impact of crop diversity on soil microbial diversity, the effect was highly variable between different studies suggesting that other context-specific drivers may be overriding the effect. Indeed, within the context of wheat-legume rotations in the Swartland, there was no significant relationship between above and belowground diversity. This is likely due to other abiotic drivers of microbial community structure such as P availability, Na and K excess, and pH, all of which correlated to microbial activity and functional richness in the soils from the Swartland rotation trial. Furthermore, literature also includes soil moisture (Marais et al. 2012) and soil organic matter (Zak et al., 2003) as important drivers of soil microbial community structure. Thus, any relationship between above and belowground diversity may be confounded by abiotic components. Agricultural practitioners wishing to boost soil microbial diversity need to consider the net-like causal structure of agroecosystem, and adopt a range of management practices facilitating the growth of microorganisms where crop rotations may be one of them. This is, however, based on the assumption that increased microbial diversity is beneficial for agroecosystem function.

The results from this thesis reveal that the link between agroecosystem diversity and function remains complicated. The meta-analysis revealed no correlation between crop or microbial diversity, and soil fertility, although the number of studies involved was low. This aligned with

the results from Chapter 2 which showed no correlation between functional and genetic microbial diversity scores, as well as no correlation between microbial diversity and soil N fertility and wheat yield. These results do not align with any one of the broader diversity-ecosystem function relationships depicted in Figure 1.1. This decoupling of agroecosystem diversity and function does provide support for the hypothesis that ecosystem function may be a product of either specific productive species (selection effect), or the facilitative interaction of multiple species (complementarity effect). For instance, a particular patch of soil may contain high microbial diversity and maintain its functionality through functional redundancy, or contain low microbial diversity and maintain functionality through specific productive species. Likewise, a particular patch of soil may have its functionality compromised by a specific pathogenic species independent of whether it has high or low levels of microbial diversity.

The one result that did buck the trend was the correlation between crop diversity and functional components including wheat yield and soil N in Chapter 2. The relationship followed a hump-shaped curve, supporting the broader ecological theory (Anderson, 2003; Nannipieri et al., 2003) and suggests that a rotation with one legume crop was more productive than wheat alone or wheat plus two legumes. Further research incorporating a variety of crops would need to be performed before a crop diversity of two crops could be claimed as optimal for production.

These conflicting results on the diversity-ecosystem function relationship challenge the broad assumption that soil biodiversity, and biodiversity in general, is beneficial for ecosystem function (Brussaard et al. 2007). Without specific knowledge about the species composition of a given agroecosystem, it is difficult to understand how the biodiversity related to function. It may be the case that for soil biodiversity, community-level microbial diversity measurements are too broad to make any statements about functionality. This highlights the need for research into the specific functions of microbial taxa within agroecosystems using techniques like pyrosequencing and other next-generation sequencing methods (Fakruddin et al., 2013). Furthermore, the spatio-temporal changes in microbial diversity and ecosystem function need to be further examined. For example, within wheat-legume crop rotations, bulk soil diversity-function measurements may produce different results to those of the rhizosphere, and the same may be true of measurements during winter and summer.

Overall this thesis supports the claim that diversifying agroecosystems through wheat-legume crop rotations is beneficial for cash crop production. The extent to which soil biodiversity and agroecosystem function, in terms of N cycling and yield, are linked may be further clarified with focused research in the following two areas; (1) the relative impact of various drivers of microbial diversity, and (2) the species composition and specific functional roles of taxa within

soil microbial communities. This thesis indicated that crop rotation (through the meta-analysis) and soil chemical characteristics are important drivers of microbial diversity, however research into the relative impact of these factors and others (biotic and abiotic) is necessary (Powell et al., 2014). For instance, it may be possible that other factors associated with crop rotations may be complicating the effect on soil microbial communities. These may include soil organic matter quantity and quality, differing tillage practices, fertilization rates, and biocide chemical usage. However, further to knowing what influences microbial communities, research on the functional role of specific microorganisms in crop rotation and other agroecosystems is necessary. For this, next-generation sequencing techniques are required to link specific keystone taxa to their functions (both beneficial and negative). These findings may increase our ability to manipulate microbial populations for agroecosystem sustainability in the future.

REFERENCES

- Alvey, S., Bagayoko, M., Neumann, G., Buerkert, A., 2001. Cereal/legume rotations affect chemical properties and biological activities in two West African soils. *Plant Soil* 231, 45-54.
- Alvey, S., Yang, C., Buerkert, A., Crowley, D., 2003. Cereal/legume rotation effects on rhizosphere bacterial community structure in West African soils. *Biol. Fertil. Soils* 37, 73-82.
- Anderson, T., 2003. Microbial eco-physiological indicators to assess soil quality. *Agric. Ecosyst. Environ.* 98, 285-293.
- Anon, 1990. Handbook of Standard Soil Testing Methods for Advisory Purposes, Compiled by the Non-Affiliated Soil Analysis Work Committee. Soil Science Society of South Africa, Pretoria.
- Azziz, G., Bajsa, N., Haghjou, T., Taulé, C., Valverde, Á., Igual, J.M., Arias, A., 2012. Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Appl. Soil Ecol.* 61, 320-326.
- Bachar, A., Al-Ashhab, A., Soares, M.I.M., Sklarz, M.Y., Angel, R., Ungar, E.D., Gillor, O., 2010. Soil microbial abundance and diversity along a low precipitation gradient. *Microb. Ecol.* 60, 453-461.
- Badgley, C., Moghtader, J., Quintero, E., Zakem, E., Chappell, M.J., Aviles-Vazquez, K., Samulon, A., Perfecto, I., 2007. Organic agriculture and the global food supply. *Renew. Agric. Food Syst.* 22, 86-108.
- Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 68, 1-13.
- Bernard, E., Larkin, R.P., Tavantzis, S., Erich, M.S., Alyokhin, A., Sewell, G., Lannan, A., Gross, S.D., 2012. Compost, rapeseed rotation, and biocontrol agents significantly impact soil microbial communities in organic and conventional potato production systems. *Appl. Soil Ecol.* 52, 29-41.
- Bird, J.A., Herman, D.J., Firestone, M.K., 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol. Biochem.* 43, 718-725.
- Bock, C.E., Jones, Z.F., Bock, J.H., 2007. Relationships between species richness, evenness, and abundance in a southwestern savanna. *Ecology* 88, 1322-1327.

- Bossio, D., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K., Ball, A.S., Pretty, J., Osborn, A.M., 2005. Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microb. Ecol.* 49, 50-62.
- Brussaard, L., De Ruiter, P.C., Brown, G.G., 2007. Soil biodiversity for agricultural sustainability. *Agric. Ecosyst. Environ.* 121, 233-244.
- Bucher, A., Lanyon, L., 2005. Evaluating soil management with microbial community-level physiological profiles. *Appl. Soil Ecol.* 29, 59-71.
- Bullock, D., 1992. Crop rotation. *Crit. Rev. Plant Sci.* 11, 309-326.
- Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. *Appl. Environ. Microbiol.* 70, 6147-6156.
- Carranca, C., Oliveira, A., Pampulha, E., Torres, M.O., 2009. Temporal dynamics of soil nitrogen, carbon and microbial activity in conservative and disturbed fields amended with mature white lupine and oat residues. *Geoderma* 151, 50-59.
- Chen, S., Zheng, X., Wang, D., Chen, L., Xu, C., Zhang, X., 2012. Effect of long-term paddy-upland yearly rotations on rice (*Oryza sativa*) yield, soil properties, and bacteria community diversity. *ScientificWorldJournal*. 2012, 1-11.
- Choi, K., Dobbs, F.C., 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *J. Microbiol. Methods* 36, 203-213.
- Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., Smalla, K., 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiol. Ecol.* 56, 236-249.
- Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92, 621-632.
- Davinic, M., Moore-Kucera, J., Acosta-Martinez, V., Zak, J., Allen, V., 2013. Soil fungal distribution and functionality as affected by grazing and vegetation components of integrated crop–livestock agroecosystems. *Appl. Soil Ecol.* 66, 61-70.
- De Vries, F.T., Thebault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjornlund, L., Bracht Jorgensen, H., Brady, M.V., Christensen, S., de Ruiter, P.C., d'Hertefeldt, T., Frouz,

- J., Hedlund, K., Hemerik, L., Hol, W.H., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., Bardgett, R.D., 2013. Soil food web properties explain ecosystem services across European land use systems. *Proc. Natl. Acad. Sci. U. S. A.* 110, 14296-14301.
- Dias, T., Dukes, A., Antunes, P.M., 2015. Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. *J. Sci. Food Agric.* 95, 447-454.
- Doi, S.A., Thalib, L., 2008. A quality-effects model for meta-analysis. *Epidemiology.* 19, 94-100.
- Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. *Diversity* 4, 375-395.
- Drinkwater, L.E., Wagoner, P., Sarrantonio, M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature* 396, 262-265.
- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A., Scherber, C., Steinbeiss, S., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91, 485-496.
- Fakruddin, M., Mannan, K.S.B., 2013. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon J. Sci. (Biol. Sci.)*. 42, 19-33.
- Ferreira, M.C., Andrade, D.d.S., Chueire, Ligia Maria de O, Takemura, S.M., Hungria, M., 2000. Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biol. Biochem.* 32, 627-637.
- Garbeva, P., van Veen, J., van Elsas, J., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu. Rev. Phytopathol.* 42, 243-270.
- Garg, A.X., Hackam, D., Tonelli, M., 2008. Systematic review and meta-analysis: when one study is just not enough. *Clin. J. Am. Soc. Nephrol.* 3, 253-260.
- Garland, J.L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* 24, 289-300.
- Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilisation. *Appl. Environ. Microbiol.* 57, 2351-2359.

- Giller, K., Beare, M., Lavelle, P., Izac, A., Swift, M., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. *Appl. Soil Ecol.* 6, 3-16.
- González-Chávez, Ma del Carmen A, Aitkenhead-Peterson, J.A., Gentry, T.J., Zuberer, D., Hons, F., Loeppert, R., 2010. Soil microbial community, C, N, and P responses to long-term tillage and crop rotation. *Soil Tillage Res.* 106, 285-293.
- Griffiths, B., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., De Ruyter, P., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *Oikos* 90, 279-294.
- Guong, V.T., Rosling, A., Alström, S., Chai, B., Högberg, N., 2012. Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, *Oryza sativa*. *Biol. Fertil. Soils* 48, 217-225.
- Hansen, A.P., 1994. *Symbiotic N₂ Fixation of Crop Legumes: Achievements and Perspectives*. Margraf Verlag, Weikersheim.
- Hardy, M.B., Strauss, J.A. 2011. Gross margin analysis of crop and crop/pasture rotation systems that are being evaluated in the long-term crop rotation trial at the Langgewens Agricultural Research Farm - 2002 to 2010. Interim report, Western Cape Department of Agriculture.
- Hawkins, H., Johansen, A., George, E., 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil* 226, 275-285.
- Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80, 1150-1156.
- Herrmann, L., Chotte, J., Thuita, M., Lesueur, D., 2014. Effects of cropping systems, maize residues application and N fertilization on promiscuous soybean yields and diversity of native rhizobia in Central Kenya. *Pedobiologia* 57, 75-85.
- Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. *Br. Med. J.* 327, 557-560.
- Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54, 427-432.
- Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 363, 543-555.

- Hooper, D.U., Bignell, D.E., Brown, V.K., Brussaard, L., Mark Dangerfield, J., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., 2000. Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks. *Bioscience* 50, 1049-1061.
- Hooper, D.U., Chapin Iii, F., Ewel, J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J., Lodge, D., Loreau, M., Naeem, S., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3-35.
- Johnson, D.W., Curtis, P.S., 2001. Effects of forest management on soil C and N storage: meta analysis. *For. Ecol. Manage.* 140, 227-238.
- Karlen, D., Varvel, G., Bullock, D., Cruse, R., 1994. Crop rotations for the 21st century. *Adv. Agron.* 53, 1-45
- Kennedy, A., 1999. Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* 74, 65-76.
- Kennedy, A., Smith, K., 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* 170, 75-86.
- Kent, A.D., Triplett, E.W., 2002. Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annu. Rev. Microbiol.* 56, 211-236.
- Kotze, I., Rose, M. 2015. *Farming Facts and Futures: Reconnecting South Africa's food systems to its ecosystems.* WWF-SA, Cape Town, South Africa.
- Larkin, R.P., 2008. Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato. *Soil Biol. Biochem.* 40, 1341-1351.
- Li, C., Li, X., Kong, W., Wu, Y., Wang, J., 2010. Effect of monoculture soybean on soil microbial community in the Northeast China. *Plant Soil* 330, 423-433.
- Lupwayi N., Rice, W., Clayton, G., 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biol. Biochem.* 30, 1733-1741.
- Manly, B.F., 2004. *Multivariate Statistical Methods: A Primer.* CRC Press, Boca Raton.
- Marais, A., Hardy, M., Booyse, M., Botha, A., 2012. Effects of monoculture, crop rotation, and soil moisture content on selected soil physicochemical and microbial parameters in wheat fields. *Appl. Environ. Soil Sci.* 2012.

- Marinari, S., Mancinelli, R., Brunetti, P., Campiglia, E., 2015. Soil quality, microbial functions and tomato yield under cover crop mulching in the Mediterranean environment. *Soil Tillage Res.* 145, 20-28.
- Mathimaran, N., Ruh, R., Jama, B., Verchot, L., Frossard, E., Jansa, J., 2007. Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agric. Ecosyst. Environ.* 119, 22-32.
- McDaniel, M., Tiemann, L., Grandy, A., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecol. Appl.* 24, 560-570.
- Murphy, D., Cookson, W., Braimbridge, M., Marschner, P., Jones, D., Stockdale, E., Abbott, L., 2011. Relationships between soil organic matter and the soil microbial biomass (size, functional diversity, and community structure) in crop and pasture systems in a semi-arid environment. *Soil Res.* 49, 582-594.
- Nair, A., Ngouajio, M., 2012. Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Appl. Soil Ecol.* 58, 45-55.
- Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *Eur. J. Soil Sci.* 54, 655-670.
- Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. *Soil Biol. Biochem.* 65, 86-95.
- Osenberg, C.W., Sarnelle, O., Cooper, S.D., Holt, R.D., 1999. Resolving ecological questions through meta-analysis: goals, metrics, and models. *Ecology* 80, 1105-1117.
- Palm, C., Blanco-Canqui, H., DeClerck, F., Gatere, L., Grace, P., 2014. Conservation agriculture and ecosystem services: an overview. *Agric. Ecosyst. Environ.* 187, 87-105.
- Powell, J.R., Craven, D., Eisenhauer, N., 2014. Recent trends and future strategies in soil ecological research—Integrative approaches at Pedobiologia. *Pedobiologia* 57, 1-3.
- Parkinson, D., Coleman, D.C., 1991. Microbial communities, activity and biomass. *Agric. Ecosyst. Environ.* 34, 3-33.

- Peet, R.K., 1974. The measurement of species diversity. *Annu. Rev. Ecol. Syst.* 1, 285-307.
- Power, A.G., 2010. Ecosystem services and agriculture: tradeoffs and synergies. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2959-2971.
- Ranjard, L., Poly, F., Lata, J.C., Mougel, C., Thioulouse, J., Nazaret, S., 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Appl. Environ. Microbiol.* 67, 4479-4487.
- Ranjard, L., Poly, F., Nazaret, S., 2000. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. *Res. Microbiol.* 151, 167-177.
- Reardon, C., Gollany, H., Wuest, S., 2014. Diazotroph community structure and abundance in wheat–fallow and wheat–pea crop rotations. *Soil Biol. Biochem.* 69, 406-412.
- Rosset, P.M., Altieri, M.A., 1997. Agroecology versus input substitution: a fundamental contradiction of sustainable agriculture. *Soc. Nat. Res.* 10, 283-295.
- Schmidt, T.M., Waldron, C., 2015. Microbial Diversity in Soils of Agricultural Landscapes and Its Relation to Ecosystem Function, in: Hamilton, S.K., Doll, J.E., Robertson, P. (Eds.), *The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability*. Oxford University Press, Oxford, pp. 135-155.
- Shannon, P.O., Weaver, W., 1969. *The Mathematical Theory of Communication*, fourth ed. The University of Illinois Press, Urbana.
- Sharma, S.K., Ramesh, A., Sharma, M.P., Joshi, O.P., Govaerts, B., Steenwerth, K.L., Karlen, D.L., 2011. Microbial community structure and diversity as indicators for evaluating soil quality, in: Lichtfouse, E. (Eds.), *Anonymous Biodiversity, Biofuels, Agroforestry and Conservation Agriculture*. Springer, pp. 317-358.
- Sileshi, G., Mafongoya, P.L., Chintu, R., Akinnifesi, F.K., 2008. Mixed-species legume fallows affect faunal abundance and richness and N cycling compared to single species in maize-fallow rotations. *Soil Biol. Biochem.* 40, 3065-3075.
- Slabbert, E., Kongor, R.Y., Esler, K.J., Jacobs, K., 2010. Microbial diversity and community structure in Fynbos soil. *Mol. Ecol.* 19, 1031-1041.
- Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing

- gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl. Environ. Microbiol.* 67, 4742-4751.
- Smith, R.G., Gross, K.L., Robertson, G.P., 2008. Effects of crop diversity on agroecosystem function: crop yield response. *Ecosystems* 11, 355-366.
- Söderberg, K., Probanza, A., Jumpponen, A., Bååth, E., 2004. The microbial community in the rhizosphere determined by community-level physiological profiles (CLPP) and direct soil-and cfu-PLFA techniques. *Appl. Soil Ecol.* 25, 135-145.
- Souza, R.C., Cantão, M.E., Vasconcelos, A.T.R., Nogueira, M.A., Hungria, M., 2013. Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. *Appl. Soil Ecol.* 72, 49-61.
- Spellerberg, I.F., Fedor, P.J., 2003. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the ‘Shannon–Wiener’ Index. *Global Ecol. Biogeogr.* 12, 177-179.
- Stefanowicz, A., 2006. The Biolog plates technique as a tool in ecological studies of microbial communities. *Pol. J. Environ. Stud.* 15, 669-676.
- Thomas, V., Kevan, P., 1993. Basic principles of agroecology and sustainable agriculture. *Journal of J. Agric. Environ. Ethics* 6, 1-19.
- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. *Science* 292, 281-284.
- Tonitto, C., David, M., Drinkwater, L., 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. *Agric. Ecosyst. Environ.* 112, 58-72.
- Torsvik, V., Goksoyr, J., Daae, F.L., 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56, 782-787.
- Torsvik, V., Øvreås, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. *Curr. Opin. Microbiol.* 5, 240-245.
- van der Heijden, Marcel GA, Wagg, C., 2013. Soil microbial diversity and agro-ecosystem functioning. *Plant Soil* 363, 1-5.
- Tummers, B. 2006. DataThief III, version 1.6. <<http://www.datathief.org/>>
- van der Heijden, Marcel GA, Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296-310.

- van der Heijden, Marcel GA, Wagg, C., 2013. Soil microbial diversity and agro-ecosystem functioning. *Plant Soil* 363, 1-5.
- van der Putten, Wim H, Bardgett, R.D., De Ruiter, P., Hol, W., Meyer, K., Bezemer, T., Bradford, M., Christensen, S., Eppinga, M., Fukami, T., 2009. Empirical and theoretical challenges in aboveground–belowground ecology. *Oecologia* 161, 1-14.
- van Elsas, J.D., Garbeva, P., Salles, J., 2002. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. *Biodegradation* 13, 29-40.
- Vitousek, P., Hooper, D., 1994. Biological diversity and terrestrial ecosystem biogeochemistry, in Anonymous Biodiversity and Ecosystem Function, in: Schulze, E., Mooney, H.A. (Eds.), *Biodiversity and Ecosystem Function*. Springer, Verlag, Berlin Heidelberg, pp. 3-14.
- Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. U. S. A.* 111, 5266-5270.
- Wagg, C., Jansa, J., Schmid, B., van der Heijden, Marcel GA, 2011. Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecol. Lett.* 14, 1001-1009.
- Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. *Science* 304, 1629-1633.
- Welbaum, G.E., Sturz, A.V., Dong, Z., Nowak, J., 2004. Managing soil microorganisms to improve productivity of agro-ecosystems. *Crit. Rev. Plant Sci.* 23, 175-193.
- Wood, D., Lenné, J.M., 1999. Why Agrobiodiversity?, in: Wood, D., Lenné, J.M. (Eds.), *Agrobiodiversity: characterization, utilization and management*. CABI Publishing, Wallingford, pp. 1-13.
- Wu, F., Yu, H., Yu, G., Pan, K., Bao, J., 2011. Improved bacterial community diversity and cucumber yields in a rotation with kidney bean–celery–cucumber. *Acta Agric. Scand. Sect. B Soil Plant Sci.* 61, 122-128.
- Yao, H., Jiao, X., Wu, F., 2006. Effects of continuous cucumber cropping and alternative rotations under protected cultivation on soil microbial community diversity. *Plant Soil* 284, 195-203.

- Yin, C., Jones, K.L., Peterson, D.E., Garrett, K.A., Hulbert, S.H., Paulitz, T.C., 2010. Members of soil bacterial communities sensitive to tillage and crop rotation. *Soil Biol. Biochem.* 42, 2111-2118.
- Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84, 2042-2050.
- Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.* 26, 1101-1108.

APPENDIX

Appendix A

Database of studies included in the meta-analysis.

Study name	Entry differentiator	Measured variable	Differentiator label	Control	Treatment	Categorical Moderating Variables			
						Variance reported	No. crops in rotation	Legume presence	Cover crop presence
Alvey et al. 2003	Rotation type & Location	Diversity	Gaya Rotation 1	1millet	1(control/1cowpea)	y [†]	2	y	n
			Kaboli Rotation 2	1maize	1(control/groundnut)	y	2	y	n
Azziz et al. 2012	Rotation length	Diversity	Short Rotation	1sorghum	2control/2pasture mix	y	6	y	y
			Long Rotation	1sorghum	2control/4pasture mix	y	8	y	y
Bernard et al. 2012	Location	Diversity & Richness	Aroostook Farm	1potato	1(control/rapeseed)	n	3	n	y
			Wood Prairie Farm	1potato	1(control/rapeseed)	n	3	n	y
Bossio et al. 2005	Location	Diversity	Luero	1maize	1(control/Tephrosia candida)	y	2	y	y
			Ugunja	1maize	1(control/Tephrosia candida)	y	2	y	y
			Teso	1maize	1(control/Tephrosia candida)	y	2	y	y
Bucher & Lanyon 2005	Rotation type	Diversity & Richness	Rotation 1	1 maize	2(control/soybean)	n	2	y	n
			Rotation 2	1 maize	4control/4alfalfa	n	2	y	n
			Rotation 3	1 maize	1control/1oats/1wheat/2clover	n	4	y	n
Davinic et al. 2013	-	Diversity & Richness	-	1cotton	1(control/millet)	y	2	n	y
González-Chávez et al. 2010	-	Richness	-	1wheat	2(control/sorghum/soybean)	y	3	y	n
Guong et al. 2012	Rotation type	Diversity & Richness	Rotation 1	1rice	1(control/maize/control)	y	2	n	n
			Rotation 2	1rice	1(control/mugbean/control)	y	2	y	y
			Rotation 3	1rice	1(control/mugbean/maize)	y	3	y	y
Lupwayi et al. 1998	Rotation type	Diversity & Richness	Rotation 1	1wheat	1(control/red clover)	y	2	y	y
			Rotation 2	1wheat	1(control/fieldpea)	y	2	y	y
Marais et al. 2012	Year	Diversity & Richness	2007	1wheat	2(control/medic)	y	2	y	y
			2008	1wheat	2(control/medic)	y	2	y	y
			2009	1wheat	2(control/medic)	y	2	y	y
Marinari et al. 2015	Rotation type	Diversity	Rotation 1	1tomato	1(control/lacy phacelia)	y	2	n	y
			Rotation 2	1tomato	1(control/white mustard)	y	2	n	y

Mathimaran et al. 2007	-	Diversity & Richness	Rotation 3	1tomato	1(control/hairy vetch)	y	2	y	y
				1maize	1(control/crotalaria)	y	2	y	y
Murphy et al. 2012	Rotation type	Diversity	Rotation 1	1wheat	4(control/canola/wheat/fieldpea)	n	4	y	n
			Rotation 2	1wheat	2(control/medic)	n	2	y	n
Nair & Ngouajio 2012	Rotation type	Diversity & Richness	Rotation 1	1tomato	1(control/rye)	y	2	n	y
			Rotation 2	1tomato	1(control/rye+vetch)	y	3	y	y
Navarro-Noya et al. 2013	-	Richness	-	1maize	2(control/wheat)	y	2	n	n
Reardon et al. 2014	-	Richness	-	1wheat	1(control/fieldpea)	n	2	y	y
van Elsas et al. 2002	-	Diversity	-	1maize	1oats/1control/1potato	n	3	n	n
			Rotation 1	1cucumber	1.33(tomato/bean/control)	y	3	y	y
			Rotation 2	1cucumber	1.33(tomato/celery/control)	y	3	n	n
			Rotation 3	1cucumber	1.33(bean/tomato/control)	y	3	y	y
Wu et al. 2011	Rotation type	Diversity	Rotation 4	1cucumber	1.33(bean/celery/control)	y	3	y	y
			Rotation 5	1cucumber	1.33(control/bean/control)	y	2	y	y
			Rotation 6	1cucumber	1.33(control/celery/control)	y	2	n	n
			Rotation 7	1cucumber	1.33(control/tomato/control)	y	2	n	n
			CLPP Rotation 1	1cucumber	2(control/tomato)	n	2	n	n
Yao et al. 2006	Rotation type & method used	Diversity & Richness	RAPD Rotation 1	1cucumber	2(control/tomato)	n	2	n	n
			CLPP Rotation 2	1cucumber	2(control/wheat)	n	2	n	n
			RAPD Rotation 2	1cucumber	2(control/wheat)	n	2	n	n
Yin et al. 2010	Tillage method	Diversity & Richness	Tillage	1wheat	2(control/soybean)	n	2	y	n
			No-tillage	1wheat	2(control/soybean)	n	2	y	n

[†] Yes (y) and No (n). [‡]Biochemical fingerprinting (BF), molecular fingerprinting (MF) and pyrosequencing (P)