An Investigation into the Use of Cover Crops to Improve Soil Health and Quality in Vegetable Crop Fields in Louis Trichardt, Limpopo Province, South Africa.

By

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Master of Environmental Science

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DEDICATION

I dedicate this work to:

My very supportive family, my beautiful wife Hulisani Mphagi, my son Tshedza Mphagi, my parents Mr Takalani Ernest Mphagi and Mrs Brendah Namadzavho Mphagi, my elder sister Konanani Mphagi and my younger brother Thikholwi Floyd Mphagi.

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ABSTRACT

This study investigated the potential of cover crops which have been green manured, to control nematodes and improve soil microbial diversity of vegetable crop fields in Louis Trichardt, Limpopo Province. For the purpose of this study, six different cover crops were initially cultivated in trial plots located in fallow vegetable crop fields and were then green manured by ploughing the biomass under when the plants were mature. These were *Lablab purpureus* (Dolichos bean), *Brassica rapa* (Mustard), *Eruca sativa* (Rocket/Nemat), *Avena sativa* (Common Oats), *Avena strigosa* (Black Oats), and *Trifolium pratense* (Red clover). All trials were conducted on the same crop field which was a fallow crop field where the last crop had been Green Pepper. The control was selected from a portion of this fallow crop field. Not all cultivations were successful as a result of unforeseen circumstances and consequently all were green manured and assessed.

Soil samples were collected from the treatment plots of three of the cover crops which were successfully cultivated soon after green manuring of the cover crops. These were then analysed to determine how green manuring from different cover crops affected nematode community profiles. Similar soil samples were collected from these three cover crops at a much later stage (after allowing for decomposition of green plant material), before analysing it for aspects of soil microbial diversity. These consisted of analyses of soil organic carbon, active carbon, soil microbial and functional diversity and enzymatic activity of the soil.

Nematode taxa were sorted into functional guilds and assigned to a colonizer-persister (cp) scale. Soil microbial species richness and abundance were measured using the Shannon-Weaver and Evenness diversity indices, respectively, while microbial enzymatic activities (ß-glucosidase, phosphatase, urease) were analysed to evaluate ecosystem functioning.

Overall results from this study showed a general domination of bacterial, hyphal and plant feeding nematodes indicative of disturbed soil and less predator and omnivore nematodes which are indicative of undisturbed soil. Concerning microbial soil activity, the control treatment (fallow crop field) displayed the highest soil microbial diversity ($p < 0.05$) compared to the cover crop treatments which had significantly lower soil microbial diversities. Of all the cover crops, the Dolichos beans (*Lablab purpureus*) had the highest microbial diversity.

In conclusion, results indicate that no substantial variances occurred in terms of nematode (parasitic and non-parasitic) community structures and population levels among samples collected from the various treatments and the soil microbial functional diversity and activity were sensitive to and varied between the various treatments.

DECLARATION

I, Mr. Mphagi Tikani Lance, hereby declare that this dissertation submitted for the degree of Master of Environmental Science in Ecology and Resource Management at the University of Venda by me, has not been previously submitted for any degree at this or any other Institution. All sources of text, data, pictures, graphs or other information from elsewhere, including the Internet, are duly acknowledged and referenced. This includes the paraphrasing of sources. Direct quotes are given in quotation marks and the source given.

Signature (student)... Date 13 August 2020.....................................

DEFINITIONS OF TERMS AND CONCEPTS

Cover crop: *"is a crop planted primarily to manage soil erosion, soil fertility, soil quality, water, weeds, pests, diseases, biodiversity, and wildlife in an agroecosystem" (*Lu et al., 2000).

Cover cropping: *"is the practice of growing pure or mixed stands of annual or perennial herbaceous plants to cover the soil of croplands for part or all of the year" (*Altieri, 1989).

Cash crop: *"a crop that is grown mainly to be sold for a cash income, rather than to be used by the farmer who grew it or those living in the area it is grown in"* (Achterbosch et al., 2014).

Green manuring: *"can be defined as the growth of a crop for the specific purpose of incorporating it into soil while green, or soon after maturity, with a view to improving the soil and benefiting subsequent crops"* (Myers, 1974)

Nematodes: *"A group of non-segmented worm-like invertebrates that occur worldwide in a wide range of habitats, including fresh and salt waters, soil, plants and animals"* (Poinar, 2018).

Microbial community: *"Multi-species assemblages, in which microbial population organisms live together in a contiguous environment and interact with each other"* (Konopka, 2009).

Microbial community structure: *"The composition of a microbial community and the relative abundance of its members"* (Reitner & Thiel, 2011).

Microbial diversity: *"The number of different species in a habitat; indicated by such measures as the number of species, genes, communities or ecosystem"* (Avidano, 2005).

Soil quality: *"the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health"* (Doran & Parkin, 1994).

Soil Fertility: *"the quality of a soil that enables it to provide nutrients in adequate amounts and in proper balance for the growth of specified plants or crops"* (Hartemink, 2006).

Environmental degradation*: "is the deterioration of the environment through depletion of resources such as air, water and soil; the destruction of ecosystems; habitat destruction; the extinction of wildlife; and pollution"* (Tyagi et al., 2014).

LIST OF ABBREVIATIONS

ANOVA	Analyses of variance
Ca	Carnivore
CI	Channel Index
cp-value	Coloniser-persister value
EI	Enrichment Index
MI	Maturity Index
PCA	Principal Component Analysis
PCA	Principal Component Analysis
PPI	Plant-Parasitic Index
PPN	Plant-parasitic nematode(s)
RDA	Redundancy Analysis
RDA	Redundancy Analyses
SI	Structure Index
SI	Structure Index
SOM	Soil Organic Matter
Spp	Species pluralism

CHAPTER 1: INTRODUCTION

1.1. Background

The primary function of cover crops is to help prevent soil degradation by water and wind erosion, which is achieved by providing soil cover and keeping it intact. Cover crops are so-called because they protect otherwise bare soil against erosion; green manures improve soil fertility. Green manuring of cover crops can improve the soil health and quality as a conservation practice and this adds benefits to the soil such as aggregate stability, infiltration, nutrient cycling, improved organic matter and biological activity when the cover crops are incorporated as soil residue (Schertz & Kemper, 1994).

Observations of the effect of cover crops on soil health, fertility and the yield of succeeding crops date back to ancient times. According to Allison (1973), incorporating cover crops into the farming system is most likely more than 3,000 years old, as shown in the Chinese manuscripts. Around 300 BC the Greeks used broad beans for this purpose (Pieters & McKee, 1938). It was also a common practice in the Roman Empire. However, until late in the 20th century, tillage technology to manage cover crops effectively, was elementary, but more effective strategies then started to appear (Hart et al., 2016).

According to Rogers & Giddens (1957), there are three different terms that are used to describe crops grown to maintain soil fertility and productivity, namely catch crops, cover crops, and green manures, but this distinction is not always applied or even practicable. A *green manure crop* is typically cultivated to assist in the preservation of soil organic matter and boost the availability of nitrogen compounds (Hartwig et al., 2002). A *cover crop* is cultivated mostly to inhibit soil erosion by providing substantial ground cover through living vegetation and roots that promote soil retention (Dabney et al., 2001). Most of the organic matter of any soil layer is in the topsoil which is often lost during soil erosion. The use of cover crops helps in the prevention of soil erosion and retaining the organic matter. Following the harvesting of economic crops, *catch crops* are cultivated to restore soil nutrients. *Catch crops* are also grown for the prevention of nutrients leaching over the winter season (Sarrantonio & Gallandt, 2003). The distinction is obscured because farmers usually have more than one goal when planting these crops during or after the main crop, and plants grown for one of these purposes may also accomplish the other two goals. The question of which term to use is not important, so for the purpose of this research, the term *cover crop* will be used (Clark, 2008).

According to Wang et al., (2002), cover crops help improve the fertility of the soil and also favour other crops by interrupting the life cycles of pest insects and help to suppress the growth of weeds. The use of cover crops can increase the annual yield production and the quality of crops by increasing the soil health and quality (Dayan & Duke, 2010). According to Thomas et al., (2003), greater soil fertility and improved water infiltration are some of the benefits that cover crops offer in a variety of cropping systems. The practice also benefits soil microbes, which, in turn, produce substances which increase soil aggregation and improves water infiltration.

1.2. Problem Statement

The over-use of pesticides and synthetic fertilizers in agriculture today is causing environmental degradation, including the deterioration of agricultural soils. Apart from the rising input cost of this farming practice, crop production has also suffered in both quality and quantity. Cover crops may offer a viable solution.

For the purpose of this study, the influence of cover crops and green manuring were investigated as alternative strategies for improving soil quality and for the control of soil-borne pests. Soil quality and health was determined using non-parasitic nematodes and soil microorganisms as biological indicators (Jansen, 2014).

1.3. Research Questions

1. How can soil organic matter be enhanced with the use of covers crops and green manuring to improve soil quality that would lower the use of synthetic chemical fertilizers in the cultivation of vegetables?
2. How can green manuring of cover crops be implemented to control parasitic and non-parasitic nematode populations and influence soil microbial activities?

1.4. Objectives

1.4.1. General Objectives

To investigate the use of cover crops to improve soil health and quality in vegetable crops in Louis Trichardt.

1.4.2. Specific Objectives

To investigate the use of cover crops through green manuring to enhance the following soil health and quality aspects and nematode populations in vegetable crop fields:

a) Soil microbial aspects
   - i) soil % organic carbon
   - ii) active carbon content
   - iii) soil microbial functional diversity
   - iv) soil microbial enzymatic activity

b) Nematode population composition

1.5. Thesis Statement

Green manuring of cover crops provides organic matter to the soil which has the potential to significantly improve the quality and health of agricultural soils by improving the physical, chemical and biological properties of the soil and which may help to reduce the numbers of harmful nematodes in the soil while improving the soil microbial activities.

1.6. Motivation

The increasing demand for food of a growing population has resulted in an intensification of mechanized farming for higher crop yields. The negative results of these practices include soil degradation. There is, therefore, a real and urgent to find viable alternatives which will produce enough food without – or at least less –environmental degradation (Linquist et al., 2012; Uprety et al., 2012). Environmental protection and sustainability have recently become a significant influence in the selection of yield-oriented production systems. The need for sustainable practices to provide long-term soil fertility and quality requires both organic and conventional production systems (Price & Northworthy, 2013). Improved soil fertility and quality enhances the ecological functioning of soil nutrient cycling, combats erosion, retains water, and creates suitable habitats for soil organisms; all this improves the physical and chemical properties of the soil for sustainable productivity and environmental quality (Dabney et al., 2001). Cover crops help to maintain and build improved levels of soil organic matter making growing high-yielding and healthy crops much easier. It helps plants to be more resistant to disease and insect pests and can better endure droughts and arid environments. By making sure that there is an adequate level of organic matter in the soil, there would be less need for the use of different types of artificial fertilizers and lime (Magdoff & Harold, 2000). Deeply rooted rotational cover crops help with the cycling of potassium, magnesium and calcium compounds as well as nitrates, all of which are usually lost to leaching

1.7. Delineation and Limitations

The emphasis of this study was restricted to investigating the potential of three types of cover crops to enhance soil health and quality aspects of vegetable crop fields through green manuring on a farm near Louis Trichardt in Limpopo Province. However, due to limited funding and time constraints, the study only covered the organic matter related soil changes and soil microorganism populations and nematode community profiling.

1.8. Underlying Assumptions

Other environmental factors such as climatic conditions may influence the soil quality results/outcomes additional to the treatments where cover crops were applied. For the purpose of this study these were considered to be insignificant.

1.9. Significance of the Study

1.9.1. Background

According to Heckman et al., (2009), the basis of an operational organic farming system is through soil health and fertility. The ideal soil makes important nutrients available to plants while also supporting a varied and vigorous biotic community that assists the soil to withstand environmental degradation. Furthermore, biological practices such as the use of cover crops for soil improvement is very crucial to reprocess and release nutrients rather than providing extraordinary measures of soluble nutrients from manufactured fertilizers. Cover crops also help to suppress weeds in the subsequent crops. Rotation of cover crops causes disruption in the life cycles of pests and weeds growth.

Cover crops such as Sunnhemp, vetch and beans, bind nitrogen in compounds which are then available to subsequent crops. The use of cover crops does not only offer nitrogen fixation, it also helps improves the soil structure and tilth due to microbial decomposition, resulting in increased soil pore space and additional cation exchange sites for nutrients (Fageria et al., 2005).

Organic matter which is added into the soil through green manuring of cover crops helps with soil improvement of intensively farmed and compacted fields and this is often seen as a much cheaper and effective method of improving soil structure (Balkcom et al., 2007). Soil with good organic matter content has shown an improved water infiltration structure during irrigation and rainy seasons. Such an advantage is particularly important in heavy soils or those with clay pans or silt layers (Blanco-Canqui et al., 2015).

1.10. Strategic Importance and Relevance

Soil is the major factor in farming, and it is therefore important to manage and protect soil to ensure maximum production of the soil while on the other hand ensuring sustainability of that soil. Farmers have realized the importance of using cover crops in improving the soil quality while on the other hand influencing production. According to Robinson et al., (1997), cover crops is considered as the most effective support of any annual cropping system that seeks to be sustainable because they help with fixing nitrogen, suppress weeds and reduce insect pests and diseases.

Plants require good tilth or quality soil for them to be able to take up adequate amounts of nutrients; this therefore means that the physical conditions of the soil significantly influence the capacity of the roots to obtain nutrients from the soil (Warncke, 2007). According to Michelle (2007), crops must be able to effectively utilize water and nutrients available in the soil and in order to achieve this, crops develop a good root system that proliferate in the top 300mm with the taproot going down to three feet. This is possible if the soil environment is favourable for the roots to grow to their full potential. Green manuring ensures that the soil is well conditioned through the incorporation of organic matter in the form of cover crops which provide the favourable environment (Thomas et al., 2003). Allowing the cover crop to attain maximum growth in the spring is important in producing thick mulch for covering the soil surface. The cover crop will need to be killed and mowed or chopped level before seeding (Michelle, 2007).

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Soil is a living resource originating on the earth's surface and only just about 10 cm of fertile soil is produced every 2000 years (Sposito, 2019). Soil quality determines the stability of an agricultural system, environmental sustainability and, eventually, plant, animal and human health. Soil is composed of both organic and inorganic materials in complex interaction (Sposito, 2019). The amalgamation of the physical, chemical and biological properties of the soil for agricultural use and environmental quality describes soil quality (Habig & Swanepoel, 2015).

According to Habig et al., (2018), food production to supply for a global population that is increasingly growing and expected to be more than 8 billion in 2050 is becoming one of the major difficulties facing humanity and in order for humanity to overcome this challenge, drastic measures for intensive food production will be required and this will result in soil degradation and loss of biodiversity and the functioning of the ecosystem. It is evident that the production capacity of the soil is dependent on its quality and conventional farming practices continues to reduce soil productivity at an alarming pace to the extent that many agricultural soils are depleted of nutrients and unable to sustain crops without artificial means (Barriuso & Mellado, 2012). These artificial additions have been very disruptive to natural ecosystems and has, over time, adversely affected soil quality and fertility (Habig et al., 2018). The use of herbicides has significantly altered the soil characteristics together with the ecological routine that it required (Kremer & Means, 2009)

Permanent soil cover, crop diversification and minimum disturbance increase and sustain organic matter which help in soil-building processes and rehabilitation of unfertile soils, and this increases yield (Pittelkow et al., 2015). High organic content also results in increased water holding capacity, increased infiltration, aggregate stability, etc. (Habig & Swanepoel, 2015).

According to Tilston et al., (2010), South African wheat farmers have gradually moved from conventional agricultural farming and adopted sustainable conventional agriculture which aims to sustain and increase soil organic matter in the soil through crop diversification, continues soil cover and minimum disturbance of the soil. According to Parr et al., (1992), many farmers opt for more environmentally sound soil rehabilitation methods, which include the use of cover crops to restore soil fertility. Cover crops are usually sown in an annual crop rotation system to shield valuable topsoil from erosion (Pittelkow et al., 2015). The primary purpose is the suppression of insect pests and weeds; but it also enhances soil fertility due to the added organic matter which aids in the retention of soil nutrients, even during the period when the cash crops are not actively growing (Snapp et al.; 2005). According to Parr et al., (1992), cover crops facilitate nitrogen fixation when green manured, protect the soil from excessive compaction, assist in weed suppression and disrupt pest and disease cycles.

Most soil research studies overlook the associated changes in the biological properties of soils and they normally put more focus on the physical and chemical properties of soils (Dominati et al., 2010). Soil microbial populations, activity and diversity are directly affected by management practices and associated changes in the soil environment (Murphy et al., 2007). This study aims to review the literature on the potential that cover crops have to improve weed suppression, nematode management, fungal diseases suppression and soil health of over-utilized vegetable crops (Habig et al., 2018).

2.2. Cover crops in vegetable production

Cover crops are mainly grown to provide soil cover either in pure stand or in association with the main crop throughout or part of the year. According to Wortman et al., (2012), cover crops are normally sown as monocultures or simple graminoid-legume bi-cultures; but there may be additional benefits of growing cover crops in mixes of greater species diversity. Derpsch (2008) noted that the cover crops most usually include rye (*Secale cereal* L.), hairy vetch (*Vicia vilosa* L.) and black oats (*Avena strigosa* Schreb), which are grown during winter; lablab (*Dolichos lablab* L.), sunnhemp (*Crotalaria juncea* L.) and cowpea are grown in summer.

Organic farming is becoming increasingly important (Greene, 2006), and demand frequently overtakes supply (Dimitri & Oberholtzer, 2009). According to Wang et al., (2002), farmers are using cover crops in order to achieve sustainable agriculture in reaction to customer pressure.

Cover crops also provide a suitable habitat for beneficial non-parasitic nematodes, suppress weeds, and act as non-host crops for parasitic nematodes in crop rotations.

2.3. Sustainable Vegetable Production in South Africa

South Africans are gradually becoming more conscious of the health and environmental concerns associated with agricultural practices. South African consumers are apprehensive about the quality and safety of vegetables that they consume. They are concerned about the consequences of fertilizers, pesticides, veterinary drugs and livestock effluent on their health and livelihood (Khwidzhili & Worth, 2016).

The modern-day organic agriculture has been largely inspired by indigenous organic agricultural methods in Africa and around the world. The traditional farming systems used globally could be referred to as ecological farming (Sposito, 2019). These farming methodologies didn't utilize any biocides; the production methodology was dependent on the natural resource base.

Organic farming excludes the use of artificial chemicals such as pesticides, fertilizers and fungicides. To improve the quality of the soil and production, organic farmers use a range of techniques that aids or sustains ecosystems and reduces pollution (Habig & Swanepoel, 2015). In plant production organic farming involves the use of cover crops, crop rotations, natural fertilizers, composting, and environmentally friendly pest control and homeopathic remedies that are approved to produce yields that do not include artificial additives (Khwidzhili & Worth, 2016).

According to Habig et al., (2018), wheat farmers in the Western Cape of South Africa have traditionally specialized in conventional agriculture, but they are increasingly adopting more sustainable conservational approaches. Crop diversification encourages soil microbial diversity and activity and improve physical and chemical soil properties by contributing more diversity in organic matter in the different soil layers (Tiffany et al., 2018).

2.4. Soil Fertility Management

Organic crop production avoids the use of artificial fertilizers, consequently soil fertility management is one of the biggest challenges to growing crops organically (Gaskell & Smith, 2007). While soil fertility is not different in organic and conventional cropping systems, there is a greater reliance on the soil biology that transforms nutrients into plant available forms, because mineral fertilizers may not be used (Mader et al., 2002).

According to Habig et al., (2015), soil microbes play a crucial role in the cycling of nutrients in ecosystems. The conversion of nutrients is accomplished by managing nutrient pools and its transformation processes and rates (Stockdale et al., 2002). Cabrera et al., (2005), highlight concerns in soil organic matter accumulation which is directly impacted by the quality and quantity of organic inputs and its effect on soil microbiology. It is widely held that soil organic matter is the foundation of nitrogen supply to crops grown organically (Magdoff & van Es, 2000; Gaskell & Smith, 2007). The importance on soil fertility has also been emphasized in the USDA National Organic Programme standards that mandate growers to develop a crop production management plan that builds the inherent fertility of soil by managing organic matter; crop rotations must comprise of cover crops which contribute to soil organic matter, effectively limiting erosion, and properly managing plant nutrients (Dayan & Duke, 2010).

Farmers have implemented a variety of organic products and management practices such as animal manures, compost and cover crops (Cogger, 2000; Gaskell, 2006); however, all these products and strategies have shortcomings. The application of manure at a period when the crop is growing will closely match nutrient needs, but can be damaging to the developing crop (Riddle & McEvoy, 2005). Well-composted materials decompose slowly, solving phosphorus loading issues but supplying smaller amounts of plant-available nitrogen (Gale et al., 2006). In contrast, some commercial organic fertilizers, such as sea bird guano, fish powder, and feather meal, regularly provide quite rapid and predictable nutrient availability. Gaskell & Smith (2007) highlight complains of farmers with regard to the prohibitive costs associated with specialty products. Furthermore, they argue that compost and manures are bulky and costly to import and apply. Cover crops, however, are often worked into rotation as a source of short-term nitrogen source (Hao et al., 2004). Both summer inter-seeded and winter cover crop mixtures have been shown to provide significant amounts of nitrogen to subsequent crops (Burket et al., 1997; Griffin et al., 2000; Sarrantonio & Malloy, 2003; Cavigelli et al., 2008). However, it is difficult to predict nitrogen availability to subsequent crops (Cabrera et al., 2005) because nitrogen mineralization of residues is determined by many factors, including residue composition, soil temperature and water content (Ruffo & Bolero, 2003b), and soil characteristics (Whitmore & Groute, 1997).

The matching of inorganic nitrogen supply with crop demand on a temporal basis remains a challenge in organic farming (Pang & Letey, 2000). Berry et al., (2002) found that while organic farming systems which use manures, composts, and cover crops have the possibility to supply large amounts of nitrogen release, is often not timed with nitrogen uptake in subsequent crops. There is a general need for a greater understanding to predict nitrogen release from various organic products and green manures so that fertilizer recommendations can be made accurately (Granatstein et al., 2010).

2.5. Cover crops overview

Herencia et al., (2008) said that cover crops are simply high numbers of plants, usually specific annual, biennial, or perennial grasses and/or legumes, growing and covering the soil surface. When a cover crop is incorporated into the soil by tilling or ploughing, it is referred to as a green manure crop (Hartwig & Ammon, 2002). Brassicas, mustards, annual cereals and annual or perennial forage grasses are the most commonly used non-legume cover crops. All these cover crops are mostly used for providing nutrients such as nitrogen remaining from preceding crops, inhibiting erosion, creating copious quantities of debris and addition of natural occurring material to the soil whilst stifling the growth of weeds (Hooker et al., 2008). The non-legume cover crops play a very crucial role as green manure in providing a different species and root system for soil building (McCracken et al., 1990). Although non-legumes cover crops cannot enable the fixation of nitrogen in the soil, they are however able to absorb large quantities from the soil which is then released into the soil when the cover crop is incorporated into the soil through green manuring. No supplementary control measures are required for the successful cultivation of most cover crops since they are not winter-hardy (Kremen & Means, 2009).

Legume cover crops supply the succeeding crop with nitrogen since they are able to assist in the fixation of nitrogen from the air as well as shielding the soil from erosion and adding organic matter (Hartwig & Ammon, 2002). Although generally more top growth equals more nitrogen fixed, the quantity of nitrogen fixed differs amongst species. Legumes species that grow for over a year have aggressive tap roots which can break up subsoil compaction (Michelle, 2007).

There are some grass cover crops with fibrous and fine root system which are well suited to holding soil in place and improving soil structure (Litterick, 2004). Grass cover crops that grow easily and are not difficult to cut down into fine pieces for green manuring and incorporation into the soil for the purpose of organic matter are deemed as suitable grass species for cover crops. Grass cover crops also accumulate large quantities of nitrogen from the soil but cannot fix any nitrogen out of the atmosphere (Kaspar et al., 2001).

Various diverse climatic conditions and cropping systems allow the successful use of annual cereal grain cover crops (Brennan & Smith, 2005). According to Sarrantonio & Gallandt (2003), winter annual cover crops establish and produce good root and top growth biomass before going dormant when seeded in late summer or autumn, then green up and produce significant biomass before maturing. Rye, wheat, and hardy triticale all follow this pattern, with some relatively small differences (Petersen et al., 2001). Brassicas and mustards release biotoxic chemicals when they break down during green manuring; these chemicals help to suppress weeds, kill parasitic nematodes and reduce disease in the subsequent crops. Because of their biofumigation characteristics farmers and researchers have shown interest in using them for agricultural and experimental purposes (Snapp, 2005; Schomberg et al., 2006).

In addition to their biofumigation characteristic, brassicas and mustards reverses soil compaction and may provide the same benefits that non-legume cover crops provide (Snapp, 2005). Forage grass like sod crops are exceptional for nutrient foraging because they control soil erosion by keeping the soil intact and because of their growing nature they provide good biomass production which suppresses the growth of weeds (Sarrantonio & Gallandt, 2003). Most perennials are grown annually for the sole purpose of cover cropping. The treatment field must be left unplanted for summer-annual grasses to accomplish a niche as a good biomass production, soil erosion control and weed suppression (Dayan & Duke, 2010).

The abundance of carbon content in grass cover crops allows for grass to break down even further than legumes, subsequent in longer-lasting residue. According to Singh et al., (2011), the higher carbon residue, the more difficult it becomes for soil microbes to break down the plant material; because the process takes longer the nutrients contained in the cover crop residue are less available to the next crop unless the next crop is planted months later after the green manuring process has been completed. Even though grass cover crops absorb excess nitrogen from the prior crop, a limited amount of nitrogen is expected to be discharged for utilisation by the succeeding crop after the grass cover crop unless planted later when the nutrients from the grass cover crop have now broken down and released for use by the follow-up crop (Rogers & Giddens, 1957). According to Sarwar et al., (1998), grass cover crops lead to increased organic matter content because of the higher carbon content and slower decomposition compared to legumes. Depending on the termination of the cover crop's maturity, the breakdown and release of carbon content of grasses and legumes after green manuring is slower as compared to the brassicas which is usually intermediate (Robison & Decker, 1997).

Legume cover crops can absorb an equal amount of nitrogen as grass cover crops, however what differentiates the two cover crops in terms of nitrogen is that legumes may quickly release it to the succeeding crop, whereas non-legume cover crops produce more organic matter which curbs erosion and subdues weeds before and after green manuring (Feaga et al., 2010). Because grasses and other non-legumes release their nutrients slowly, they are mostly not substantial sources of nitrogen for the cropping system; however, they prevent soil erosion and subsequently preserve the soil organic matter by retaining excess soil nutrients (Monfort et al., 2007)

2.6. Cover crop benefits

Cover crops provide a range of benefits and ecosystem functions and thus have been a fundamental part of agricultural conservation for many years, (Sarrantonio & Gallandt, 2003; Fageria et al., 2005; Cherr et al., 2006). According to Paine & Harrison (1993), the earliest use of cover crops date back to 500 BC, where Chinese records reveal an understanding of its value as fertilizer.

Clovers and other legumes filled traditional fallow periods in turnip-grain rotations in the eighteenth century in Europe and winter cover crops were common in cropping systems in the early twentieth century in North America (Pieters, 1917). Nevertheless, the widespread availability of commercial synthetic fertilizers fostered a decline in the use of cover crops in most industrial countries, as fertility systems based on soluble nutrient sources replaced cover crop practices in most cropping systems (Galloway & Cowling, 2002). Organic cropping systems (where mineral fertilizer use is prohibited) have given rise to a renewed interest in including cover crops for the purpose of soil fertility management and enhanced crop production (Dayan & Duke, 2010).

Cover crops are grown for the primary purpose of soil protection from erosion, suppress diseases causing pest, prevention of water loss, suppression of weeds and to enhance productivity between phases of regular crop production or inter-seeded between rows of cash crops (Hartwig & Ammon, 2002; Sarrantonio & Gallandt, 2003). These cover crops are then terminated and incorporated into the soil through green manuring to help enhance the organic matter content of the soil. Inclusion of cover crops in crop rotations provides on-farm benefits and environmental services, including reduced soil erosion and runoff, improved nitrogen cycling, weed and pest control, enhanced soil fertility and quality, and increased crop yields (Fageria et al., 2005; Snapp et al., 2005; Cherr et al., 2006). However, benefits of inclusion of cover crops in rotation vary among different soils types, crop rotations, environmental variables, type and species of cover crop, and management schemes (Fageria, 2007).

According to Habig et al., (2018), the combination of zero tillage and the inclusion of cover crops in agricultural practices increase healthy connections and the numbers of non-parasitic nematodes such as omnivorous and predatory species. Zero tillage also results in a greater soil microbial diversity and speeds up the mineralization of carbon, nitrogen and phosphorous.

2.6.1. Weed Suppression

Cover crops decrease the opportunity of weeds to establish themselves in the soil by occupying space and thereby shading the soil (Cavigelli et al., 2008). The soil-loosening results of deep-rooting green manures decrease weed populations that flourish in compressed soils. Cover crops such as millet, rye, or Sudan grass's most crucial purpose after green manuring is to provide weed control, improve soil tilth, and add organic matter (Moller et al., 2008). Cover crops are an effective management option for weed control and suppression within agricultural systems. According to Holness et al., (2008), the competition for nutrients, water, light and modification of the microclimate by cover crops is one means of weed control. Isik et al., (2009) cited this mechanism in discovering that ryegrass, oats, hairy vetch, and common vetch cover crops grown over winter or fallow periods reduced total weed biomass by 86-90 % compared to winter fallow alone. Summer inter-seeded cover crops can effectively reduce weed biomass but competition with main crops and yield reduction can be a problem (Holness et al., 2007). When the stalks of killed cover crops are left intact, their mulches last longer, which helps in providing weed control, especially for summer-grown vegetables. However, relay planted cover crops planted between main crop rows after crop establishment can provide effective weed suppression without reducing yields (Vanek et al., 2005).

Weed emergence and germination may also be controlled through the release of allelopathic chemicals (Weston, 1996). Rehman et al., (2008) suggested that cover crops, such as wild radish, contain ten identified glucosinolates, or possible allelopathic elements, which contribute up to 35% decrease in weed biomass in a wheat rotation. Research demonstrated that nitrogen availability can also influence weed germination and biomass (Lawson, 2010). Kumar et al., (2008) found that *Fagopyrum esculentum* (buckwheat) residues suppressed *Capsella bursa-pastoris* (shepherd's purse) growth and emergence through nitrogen immobilization. Similarly, Sainju et al., (2010) reported that higher nitrogen availability in hairy vetch resulted in increased weed biomass. By contrast, hairy vetch mowed at flowering and left on the surface prior to planting cash crops reduced weed biomass by 40% (Campiglia et al., 2010). Organic matter content provides physical barriers to weed development (Campiglia et al., 2010). Sustainable agriculture has adopted the use of living mulches and allelopathic cover crops as an important weed suppression control measure (Bending et al., 1990). This is because allelopathic plants prevent the development of other plants around them by discharging allelochemicals which function as natural toxins for weeds (Sainju et al., 2010). According to Crandall et al., (2005), small grains like summer annual forages interrelated to sudan grass, sorghum and rye cover crop, are among the plants that suppress weeds growth. Magdoff & Weil (2004) established that termination and incorporation of allelophatic cover crops into the soil by green manuring provides substantial weed control in no-till cropping systems. Living mulches suppress weeds throughout the growing season by competing with them for moisture, space, light and nutrients (Kremen & Means, 2009).

2.6.2. Improved Soil Health

Improved soil health is the basis of a successful organic agricultural farming system. Plants receive important nutrients from fertile soil, and this leads to a diverse and active biotic community that prevents soil degradation. As opposed to the use of high amount of insoluble inorganic fertilizers, more importance is placed on the use of cover crops and other biological practices to enhance, recycle and release nutrients into the soil. The focus is on the impact of soil microbes and organic matter content on systems for managing and maintaining soil fertility for crop production (Singh et al., 2011).

2.6.3. Organic Matter Content

The termination and incorporation of cover crops in the soil through green manuring leads to the addition of organic matter into the soil (Kumar & Pandey, 2001). Decomposing organic matter in the soil produces microorganisms that help binding together the soil particles, increase microbial communities and favour non-parasitic nematodes in the soil. Soil with good organic matter is well-aggregated and tills easily, it is well aerated, and has a high water infiltration rate (Moonen & Barberi, 2008). Increased levels of organic matter also influence soil humus in the soil and provides a wide range of benefits to crop production and cover crop mixtures are very significant in crop rotation systems since they benefit the cropping system by replacing organic matter lost during annual cultivation. (Robinson & Decker, 1997).

Soil organic content is typically used as the major indicator of soil quality and health (Doran, 2002) because of the role it provides in nutrient cycling, soil tilth, biological activity, and water availability (Magdoff & van Es, 2000). Increased organic matter in soil augments the soil's capacity to store nutrients and water and it also enhances the soil cation exchange capacity, hence improving the capability of the soil to hold macronutrients, such as potassium, calcium and magnesium (Thomas et al., 2003). Studies show that soil with cover crop residues have large amounts of carbon (Kuo et al., 1997, Sainju et al., 2005; Fortuna et al., 2008) and this is as an outcome of improved soil organic matter (Nyakatawa et al., 2001).

The increase in organic matter promotes the soil water holding capacity in sandy soils and advances water percolation in heavy, clay soils. Cereal cover crops increase soil organic matter levels relative to other cover crops by supplying better carbon contributions in the form of plant biomass (Sainju et al., 2000; Ding et al., 2006). Legume cover crops can preserve soil organic matter if the biomass is large enough. According to Fortuna et al., (2008), a constant maize-hairy vetch cropping system improved total soil carbon and nitrogen, compared to management without hairy vetch because of greater residue inputs. Bakht et al., (2009) established that soil organic carbon improved in a cropping system with legume cover crops as compared to a no legume, fertilizer-based system, which was probably because of better carbon input from plant biomass.

2.7. Nematodes

Nematodes are roundworms, mostly microscopic and the most abundant of all metazoan animals (Bird et al., 2003; Decraemer & Hunt, 2006; Davies et al., 1991). Thus far 15 000 species have been identified and it is estimated that there are half a million extant species (Poinar, 1983). In addition, populations count in the billions and trillions, which means that they have a large impact on the vegetation, directly and indirectly.

Compared to the other seven feeding groups namely bacteria, fungi, substrate feeders, predators, eukaryote feeders, animal parasites and omnivores (Yeates et al., 1993. identified in soil ecosystems, the plant-parasitic nematodes have received the most attention due to their direct economic impact on agriculture. Most of the nematode species feed on plant roots and consequently weaken the plants, and in the process, they usually cause infections through feeding wounds. According to Perry et al., (2009), plant-parasitic nematodes can cause damage to the crop by damaging plant tissues, including the retarded growth of cells, which is perceived as underdeveloped shoots, or extreme growth, such as root galls, swollen root tips or abnormal root branching.

It is common in nematode communities that comprise of diverse species that no particular species will dominate. In conventional cropping systems, the soil environment is very conducive for the development of pest nematodes as there is abundant food (Wesemael et al., 2011) and because of these favourable conditions there is a rapid development of plant parasitic species and plant diseases and this results in yield losses (Karssen & Moens, 2006). Plant-parasitic nematodes are one of sub-Saharan Africa's most serious constraints in crop production (Fourie et al., 2015). According to Jones et al., (2013), intensive monoculture in agricultural crop production worldwide is becoming exposed to damages caused by plant-parasitic nematodes such as the Meloidogyne spp. and this has led to the development of innovative, ecologically sound and efficient solutions for managing these pests. Using cover crops in a crop rotation system improves fertility, physical and chemical properties of the soil and this helps improve the productivity of the subsequent crops by reducing pressure of pests and pathogens as they serve as a non-host of nematodes (Ratnadass et al., 2012).

According to Monfort et al., (2007), the control of weeds, nematodes, bacterial and fungal diseases are some of the optimistic outcomes that cover crops have in relation to integrated pest management of the cover crop species, brassica species have the ability to generate secondary metabolites that contains sulphur such as isothiocyanate (Lazzeri et al., 1993; Brown & Morra, 1997). Larkin & Griffin (2007) discovered that green manuring of certain brassica species suppresses soil-borne pests and diseases. Fungicidal compounds like the isothiocyanate which are released during the green manuring of the *Brassica napus* can suppress certain wheat diseases (Kirkegaard et al., 1996; Sarwar et al., 1998).

Cadet & Spaull, (1985) established that the reduction in the length of the stalks in South African sugarcane plantations, which results in a lower yield is mainly caused by parasitic nematodes. The increase in the population densities of *Xiphinema elongatunt* and *X vanderlindei*, the dominant *ectoparasites* between treated and untreated nematicide plots determined the length of the sugarcane stalks.

According to Balkcom et al., (2007), the inception of nematode problems is usually curbed by increasing biological diversity of cover crops during cropping. This is because of maintaining soil ecological equilibrium and an enhanced, healthier soil structure with developed organic matter (Monfort et al., 2007). It becomes very difficult to eliminate a nematode species once it has established in the soil. However, the planting of some cover crops before or after a parasitic nematode hosting crop is planted, can highly reduce the resident parasitic nematode population (Wang et al., 2002).

The planting of susceptible cover crops in soil without prior nematode infection will not be problematic provided the species is not brought up together with the seed, transplants or machinery during planting. Countless studies show that cover crops can advance nematode antagonistic actions. Linford (1937) already mentioned that integration of organic matter into the soil could offer an environment that favours crop development and nematophagous fungi and a sequence of biological events may be involved. The decomposing organic matter is a significant occurrence since the bacteria which multiply after organic matter integration develops as a food base for microbiovorous nematodes, which becomes food source for nematophagous fungi (Bouwman et al., 1994). Investigations conducted by Li et al., (2010), under greenhouse conditions determined that changes in nematodes community in association with manure application and chemical fertilizers reduced educed the maturity index (MI) and channel index (CI), although the enrichment index (EI) increased with soil nutrients. However, little is known about long-term changes in nematode communities in association cover-crop management.

According to Neher (2001), agricultural production and human health is affected by several harmful species of nematodes. Nevertheless, there are several species of nematodes that parasitizes or preys on harmful nematodes, thereby playing a vital role in the functioning of ecosystems. The normalization of the rates of decomposition and nutrient mineralization in the soil which leads to variations in the abundance of bacterial and fungal feeding nematodes, is caused by the bacterial feeding nematodes which control the microbial activity and this indicates the fluctuations in the decay path and energy movement (Bongers & Bongers, 1998; Ferris et al., 2012).

Table 2.1: Classification of the different nematode genera into functional groups (Yeates et al., 1993, Bongers & Bongers 1998, Neher 2001).

Functional group	Selected examples of nematode genera belonging to functional groups
Plant feeders or plant-parasitic nematodes	Helicotylenchus spp., Heterodera spp., Meloidogyne spp., Pratylenchus spp.
Bacterial feeders or bacterivores	Cephalobus spp., Caenorhabditis spp., Plectus spp., Rhabditis spp.
Fungal feeders or fungivores	Aphelenchus spp., Aphelenchoides spp., Diphtherophora spp., Leptonchus spp.,
Substrate ingesters	Daptonema spp., Eumonhystera spp., Neodiplogaster spp
Animal predators	Diplogaster spp., Labronema spp., Laimaphelenchus spp., Mononchus spp., Nygolaimus spp., Seinura spp.,
Omnivores	Nematodes belonging to the order Dorylaimida

2.8. Nematodes and Soil Microbial Diversity

The co-existence of nematodes and microorganisms in a habitat stresses the importance of studying the potential relations between these two groups. According to Scheu et al., (2005), the soil food web is exceptionally multifaceted and at the same time it is most often than not poorly understood. The relationship between the soil invertebrates and microorganisms is both direct and indirect. According to Jansen (2014), the characteristic of an indirect relationship are habitat establishment and resources whereas the direct relationships include predator-prey interactions. The two most significant compartments associated with the soil food web of nematodes include firstly nematodes that add to the bacterial energy channel through plundering on bacteria as the main consumers and secondly nematodes which promote the fungal energy channel such as the fungivores and other associated predators (Ferris et al., 2001; Scheu et al., 2005). According to Briar et al., (2007), because of their proximity to microbes, it is assumed that nematodes are affected by numerous influences which also impact microbial communities. According to Jansen (2014), these effects include the abundance of bacterivores and fungivores as well as certain nematodes trophic groups and escalate the availability of microbial biomass and nutrients. An increase in the population of bacterivore nematodes is concurrently influenced by the rise of organic matter which leads to an upsurge of microbial biomass in the amended soil.

According to Habig et al., (2018), non-parasitic nematodes and soil microbial communities contribute to the soil food web stability by assisting critical soil biogeochemical processes in soil ecosystems such as the recycling of carbon-containing substrates, nitrogenous compounds, and transforming soil organic matter into mineral and organic nutrients due to their activity, diversity and wide feeding range and these are in turn made available for use by plants, influencing plant growth and crop productivity. Nematodes and microbial communities are ideal biological indicators of soil quality changes because they react quickly to disturbance and stress caused in the environment because of their in-depth connection (Sharma et al., 2010).

Furthermore, studies done by (Briar et al., 2007; Jansen, 2014) illustrate that the interaction between microorganisms and nematodes declined in an arid season and was by a rise in nematode bacterivore numbers months *after* the increase in microbial biomass. This is an indication of the effect that non-parasitic nematode communities have on microbes. However, Yeates et al., (1999) stated that an escalation in microbial biomass associated with an increase in predacious rather than bacterivorous nematodes can be as a result of the predacious nematodes affecting other non-parasitic nematode populations. Due to the variations shown in different theses, it is therefore important that the existence and diversity of nematodes existing in soils are in future compared with those of microorganisms (Jansen, 2014).

2.9. Cover crop management

Farming objectives determine the cover crop management scheme to be implemented (Sustainable Agriculture Network, 2007). Goals and ground types affect what species are most suitable to a given rotation and environment (Snapp et al., 2005).

2.9.1. Integration of cover crops in crop rotations

The process of determining spatial and temporal niches where cover crops can be integrated into crop rotations can be the highest challenge to meaningful over-crop use (Snapp et al., 2005). There are a number of considerations for identifying where and when cover cropping is possible, including seeding method, weather restrictions, soil conditions, cover crop growth habit, method of kill and subsequent bed preparation, as well as outcomes in economic crop yield and growth (Sustainable Agriculture Network, 2007; Cherr et al., 2006). Vanek et al., (2005) explain that cover cropping is limited in vegetable production because of frequent tillage, complex rotations, and continuous cropping for harvest.

Non-simultaneous cover cropping, relay planting, and intercropping as living mulch between crop rows are regarded as options in crop rotations. Relay planting is a system where cover crops are planted in standing cash crops, allowing early establishment of cover crops without negatively impacting the cash crop growth (Sarrantonio, 1992). Winter cover crops planted after cash crop harvest are most common in temperate climates, because full-season or summer cover crops represent income loss from the field that is not cropped for an entire growing season (Griffin et al., 2000).

According to Cherr et al., (2006), the fallow period of a rotation when weather is not favourable for production of cash crops, is when cover crops are usually planted. Winter grown cover crops are grown without sacrificing ground for cash crop production. The establishment of cover crops can be difficult in temperate climates, though, because of low temperatures at the time of sowing and significant loss to winterkill (Carrera et al., 2004). Several studies list rapid germination and establishment, extensive rooting systems, good winter-hardiness, and early spring growth as characteristics for successful winter cover crops in cool, temperate climates (Nelson et al., 1991; Creamer et al., 1997; Delate et al., 2008). However, variability in annual weather conditions can result in inconsistent cover crop biomass yield between years, despite cover crop vigour and cold-tolerance (Burket et al., 1997; Odhiambo & Bomke, 2000; Rinnofner et al., 2008). The literature shows that promising winter cover crops for temperate climates include monocultures or combinations of barley, rye, ryegrass, wheat, hairy vetch, crimson clover, and red clover (Nelson et al., 1991; Creamer et al., 1997; Carrera et al., 2004).

Cover crops are often inter-seeded with the cash crop and kept as a living ground shield over the cropping season (Hartwig & Ammon, 2002). Soil improving cover crops can be grown without reducing land for cash crop production and it can potentially control weeds, which is why relay or simultaneous planting of cover crops with a cash crop is attractive (Sarrantonio & Gallandt, 2003). Cover crop species and timing of planting in relation to crop establishment has an impact on cash crop yield because of competition for nutrients, water, and light, and modification of growing conditions (Brainard & Bellinder, 2004). For example, annual cover crops planted simultaneously with maize reduced early growth and subsequent yield by decreasing nitrogen availability to the crop (Biazzo & Masiunas, 2000). However, Negrini et al., (2010) found that intercropping white lupine with lettuce did not affect lettuce performance when planted at the same time. In the same study, black oats or cowpea sown 20, 40, and 60 days prior to lettuce were found to negatively affect lettuce growth and yield because of competition for sunlight. Relay planting cover crops can benefit crops by contributing nitrogen to intercropped or subsequent cash crops without reducing crop growth and yield (Jeranyama et al., 1998). Additionally, wide row spacing and rapid growth habits of some crops, such as pumpkins, make some crop systems better suited to intercropping than others (Vanek et al., 2005). For these reasons intercropping schemes are specific to site and cropping system.

2.9.2. Cereal, legume and herb cover crop blends

The reduction of stand failure, biomass productivity enhancement and the prevention of nitrogen immobilization are accomplished using mixed cereal-legume cover crop blends (Sustainable Agriculture Network, 2007). Numerous studies have established that monocultures have decreased yield stability compared to mixtures, because single strand species have a narrow range of lenience to adverse environmental circumstances than when grown in conjunction with other species (Creamer et al., 1997; Gaskell, 2006). In some cases, the cereal crop enhances winter hardiness by reducing exposure to harsh weather for the legumes. Furthermore, performance of a legume can also be enhanced by a cereal crop when grown in bi-culture. For example, the support from rye plants prevented lodging in winter pea in one study (Karpenstein-Machan & Stuelpnagel, 2000). Moreover, cereal crops, such as rye, can provide structure for sprawling crops like hairy vetch, thereby increasing use of solar energy and biomass (Sustainable Agriculture Network, 2007).

Cover crop blends that include cereal and legume species improve nitrogen cycling and nitrogen availability to subsequent crops. This is because cereals are capable of recycling residual soil nitrate after fall harvest to minimize leaching while legumes fix atmospheric nitrogen, which increases nitrogen concentration in residues (Clark et al., 1997). But in one rye-winter pea mixture, high residual soil nitrogen decreased winter pea establishment and vigour because rye exhibited a greater competitive advantage in high nitrogen conditions (Burket et al., 1997). In some cases, the nitrogen concentration of a cereal in mixture with a legume is higher than when it is grown alone. Evans et al., (2001) observed that cereal residue had a 28% higher nitrogen concentration when grown in mixture with red clover than when cropped alone.

Cereal and legume cover crop mixtures frequently produce better biomass and accumulate more organic matter than when grown in monoculture (Ranells & Wagger, 1996; Clark et al., 1997; Sainju et al., 2005). For example, Lu et al., (2000) found that hairy vetch accumulated 4.67 to 5.75 Magnesium per hectare of dry matter while a rye-hairy vetch mixture produced 8.95 to 11.17 magnesium per hectare in one study, rye-hairy vetch bi-culture dry matter was no different than that of pure rye (Cline & Silvernail, 2001). In the same study, the bi-culture accumulated significantly more organic matter than a pure cereal crop, because of the high organic content of the residues in the blend. While mono cropped cereals, such as rye, can produce a lot of organic matter following incorporation, cereal-legume bi-cultures can supply organic matter to subsequent crops (Kuo & Sainju, 1996).

2.9.3. Cover crops and green manuring

According to Pieters (2006), green manuring is defined as the incorporation of crop biomass into the soil while the crop is at its flowering stage, as a supplement of organic matter to the soil either where it is cultivated, or when it is imported from another site. He further pointed out that the terms “cover crop” and “green-manure crop” are often identical in practice. Besides green manuring, a cover crop can also be used for other purposes. When a cover crop is seeded for oil improvement, it can be turned into a green-manure crop once it has served its purpose as a winter cover.

The basic structure of the environment and the ecosystems can be changed directly by farming activities (Zaccheo et al., 2016). After years of agricultural practices, the physical, chemical and biological aspects of cultivated soils start showing some changes associated with nutrient availability, aggregate steadiness and cation exchange capacity (Carneiro et al., 2009), and organic matter is an important indicator to detect these imbalances in the soil and are negatively shaped by people's actions (Pereira et al., 2010).

Ploughing disturbs the soil structure intensively and also brings forth a decrease in organic matter and this modifies the balance of pre-existing organic matter in the system (Silva et al., 1994). This is aggravated by soil erosion, decline in soil fertility and compaction (Fageria, 2007). In the quest to finding sustainable agricultural practices, Calegari (1995), Silva et al., (1999) discovered that the use of cover crops enhances the quality of agricultural soil, and this improves crop production (Singh et al., 2011; Pikul et al., 1997).

The upsurge in soil organic matter, decline in erosion and evapotranspiration produced by plant residue, nutrient cycling and disruption of compacted soil layers, are all advantages of cover crops (Calegari, 1995). In addition to this, cover crops also benefit the cropping system with their ability to decrease the incidence of pests and diseases while improving microbial activity; it also helps in the suppression of weed plants (Fageria et al., 2005).

There are studies that show the practice of cover crops in the numerous civilizations that inhabited the Earth (Pikul et al., 1997; Fageria, 2007). The use of *Avena sativa, Fagopyrum esculentum* and *Oryza sativa* to incorporate organic matter into the soil through green manuring was practiced by pioneer settlers in some parts of North America (Fageria, 2007). In the fourth century BC, *Vicia faba* L. and *Lupinos* sp where cultivated by Greek and Roman civilizations with the main aim being to increase soil fertility and offer some of the nutrients essential to crops as food sources (Singh et al., 2011). During the 18th century, agricultural productivity was completely reliant on natural resources (Pikul et al., 1997). According to Fageria (2007), in the 1960's agricultural trends changed because of the increase in the implementation of intensive cropping structures, the quantities of chemical fertilizers and the high demand for nutrients by modern cultivars.

According to Creamer et al., (1997), green manured cover crops are also referred to as fertility building crops because they were developed for the benefit of the soil. With the increase in the use of pesticides and fertilizers, conventional farming systems largely rejected the use of cover crops and, although cover crops play a vital role in the cropping system, they are still underutilized in the agricultural system to date (Andow, 1988). However, according to Frye et al., (2000), the importance of reducing environmental impacts and the protection of human health in all faming systems has led conventional agricultural system to develop an interest of growing cover crops. Cover crops are not grown for sale but are produced solely for soil fertility improvement and other benefits to cash crops (Creamer et al., 1997).

According to Pieters (2006), the preservation of the nitrogen supply is a significant objective of green manuring and the preservation of good tilth is among some of the significant benefits of green manuring cover crops because of their particularly as regards organic matter. Soil with organic matter from green manures, as well as crop residues, play a significant role in good tilth and in these green manures, as well as crop residues, play an important part (Schertz & Kemper, 1994). Green manuring also provides the soil with a good quantity of nitrogen and aids in improving the physical structure of the soil. Pieters (2006) further explained that when cover crops are broken down during green manuring, they release carbon dioxide which has a distinctive dissolving effect on soil minerals and increase the solubility of phosphates.

CHAPTER 3: MATERIALS AND METHODS

3.1. Site Description

The study was an on-site investigation, carried out at a farm in Limpopo Province, which is located 5 kilometres east of Louis Trichardt next to Kutama Sinthumule Correctional Centre. The GPS coordinates are 23°04'10" S and 29°55'28" E. According to Mucina & Rutherford (2006), the soil is classified as reddish or brown sandy to loamy soil. The climatic condition in the study area ranges from 18 °C to 28 °C with an average of 25 °C. The summers have a high number of sunshine hours with the occasional afternoon thunderstorms. Winters usually last from June to August and are dry, mild and mostly frost free. The average precipitation for the study area ranges between 450mm to 800mm in November to March and approximately 75% of the rainfall occurs in the months of January and February. Environmental variables that may impact the outcomes of the results collected were considered and identified carefully. The cover crop treatment plots were replicated to meet statistical requirements for a fair representation of the factors involved.

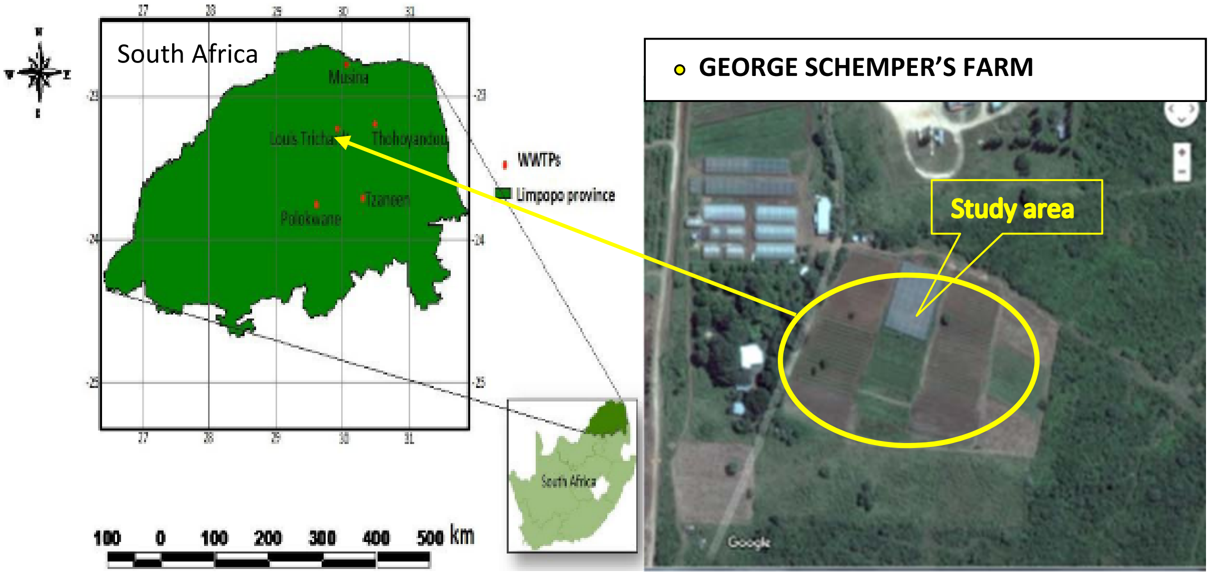

Figure 3.1: Maps showing the study area. (Shamuyarira, 2014), (Google Earth)

3.2. Research Experimental Design and Treatments

Table 3.1: Dates for sampling and field activities.

Activities	**January-November 2019**
Planting	19 January 2019
Green Manuring	25 June 2019
Sampling for Nematodes after green manuring	30 June 2019
Sampling for Soil fertility after green manuring	27 October 2019

The trials were conducted over a period of 10 months from January 2019 to October 2019 (Table 3.1). The experimental design was a randomized complete block prepared in triplicate with six cover crop treatments. The treatment plot was 3000 m^2 in size with each cover crop occupying a space of 500 m^2; each treatment was replicated three times. The cover crop treatments were monocultures of *Brassica rapa* (mustard), *Eruca sativa* (Rocket or Nemat), *Lablab purpureus* (Dolichos beans), *Avena strigosa* (Black Oats), *Trifolium pratense* (Red Clover) *and Avena sativa* (Common Oats). The chosen cover crop species represents a mixture of plant families corresponding to different plant functional groups (i.e. Poaceae, Brassicaceae and Fabaceae). Prior to establishment of the treatment plots, the site was mouldboard ploughed and the seedbed was prepared using a cultivator. Cover crops were broadcast seeded by hand and seeds were incorporated into the soil with a hand hoe. Because of their high replicability field experiments were considered the most appropriate. To meet statistical requirements for adequate representation, all treatments were replicated three times. All cover crops were then chopped up before they were ploughed in and incorporated into the soil shortly after reaching maturity and flowering; this process is called green manuring.

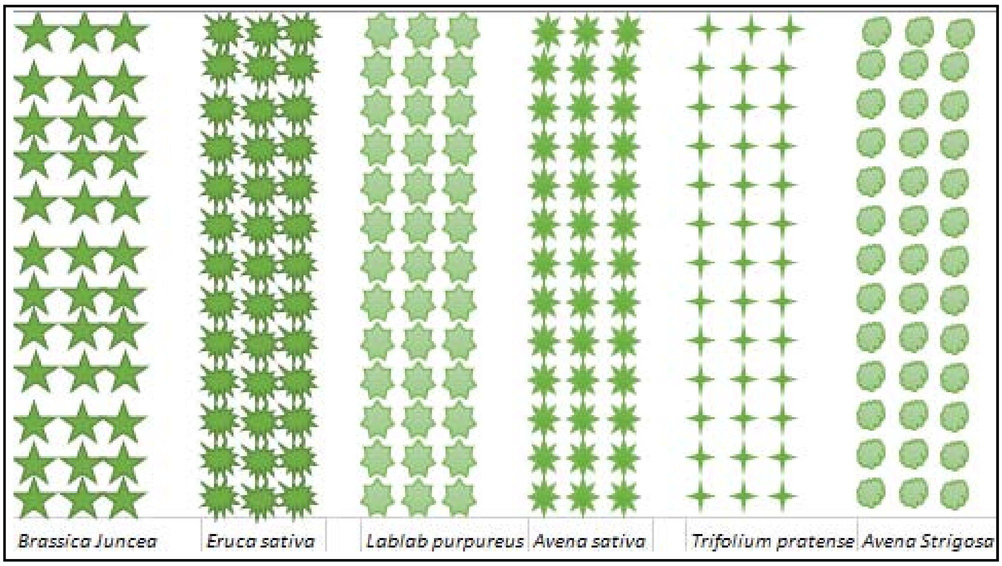

Figure 3.2: Illustration of experimental plot layout

Figure 3.3: Green manuring of cover crops (Photograph by T.L. Mphagi)

3.3. Sampling and Analysis

3.3.1 Nematode Functional Groups

Soil samples of three (*Lablab purpureus* - Dolichos Beans, *Eruca sativa* - Rocket/Nemat and *Brassica rapa* - Mustard) of the original six the cover crop treatments were collected a few days after green manuring and sent to a laboratory at the Agricultural Research Council at Roodeplaat in Pretoria. The sieving sugar centrifugal method was used to extract both the plant-parasitic and the non-parasitic nematodes from the soil samples (Marais et al., 2017; Swart & Marais, 2017). Following extraction of nematodes from the soil samples, calculations were done using a stereomicroscope at 110 x magnification and allocated into functional groups. Bacterivores, predators, fungivores and plant-parasitic nematodes encompassed these functional groups based on specific morphological features. A 4% formaldehyde solution was used to kill and fix the nematodes (Bridge & Starr, 2007). Scientists from the National Collection of Nematodes, Biosystematics Division at the ARC laboratory, identified and enumerated the extracted nematodes using the classification as given in Swart et al., (2017) and Van den Berg et al., (2017).

To allow the identification of nematodes to genus level, provisional slides were arranged by fishing numerous nematodes from the several functional groups throughout the calculation procedure and placing each sample in a drop of tap water on a microscope slide. In accordance with Ferris & Matute (2003), a clear fingernail polish was used to seal the microscope slide after the cover slide was placed on the sample. A light microscope was then used to identify the parasitic and non-parasitic nematode individuals to genus level as described by Heyns (1971) based on the morphological characteristics, which included basal bulb position and structure; lip region (head) structure and position; tail shape; amphid presence or absence; spinneret absence or presence; stoma (pharynx) shape and size; vulva position (females); and bursa presence or absence (male).

Functional guilds as defined by Yeates et al., (1993) were used to sort the extracted nematodes. The following colonizer-persister (cp) scale of 1-5 linear was used to assign the various soil nematode taxa according to their r- and K-characteristics, as defined by Bongers & Bongers (1998):

- cp-1: short generation time nematodes capable of explosive population growth under food-rich conditions. The *rhabditids*, *diplogasteri* pollution-induced strss.ds and *panagrolaimids* form this group. These are moderately tolerant to pollution-induced stress nematodes.
- cp-2: short generation time and high production rate nematodes that do not form dauer larvae. The smaller *tylenchids*, fungal feeding *aphelenchoids* and *anguinids* and bacterial feeding *cephalobids*, *plectids* and *monhysterids*.
- cp-3: longer generation time nematodes that are relatively sensitive to disturbances. Fungivores, bacterivores and carnivores.
- cp-4: the small *dorylaimids* and large non-*dorylaimids*, nematodes characterized by a long generation time which are sensitive to pollutants, composed of large carnivores and the bacterial feeding Alaimidae and Bathyodontidae, smaller *dorylaimids* and plant feeding *trichodorids*.
- cp-5: nematodes with low metabolic activity, large *dorylaimid*, very sensitive to pollutants and their disturbances due to a permeable cuticle.

The cp-classes were given according to Bongers & Bongers (1998), with most of the bacterivores belonging to cp-1 and cp-2. The fungivores to cp2 and cp-3 and cp-4, omnivores to cp-5 and the predators to cp-5. Based on the cp-values of the nematodes a Maturity Index (MI) was calculated as a tool to indicate the level of disturbance in the soil sampled. The MI ranges from 1 (disturbed) to 4 (undisturbed) (Bongers, 1990). Finally, the faunal profile was compiled from work done by Sieriebriennikov et al., (2014) and the analyses done by using the Ninja program.

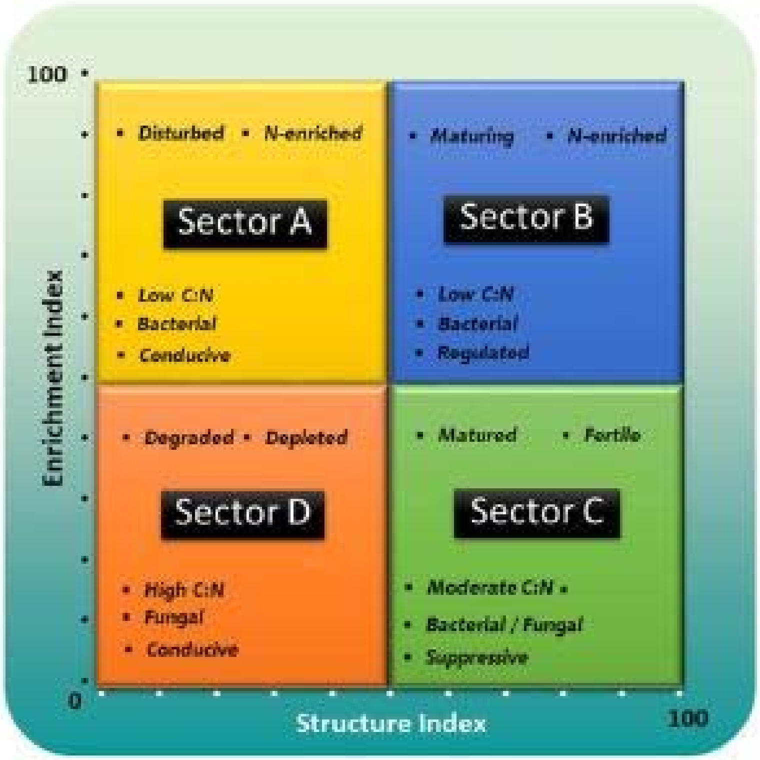

Figure 3.4: Ninja program (Sieriebriennikov et al., 2014)

Based on the reproductive rate and associated characteristics of nematodes, Bongers (1990) allocated terrestrial nematodes on a scale from colonizers to persisters (cp-values). Functional guilds of different taxa where categorised by Ferris et al., (2001) (where members respond similarly to food web enrichment and environmental perturbation/recovery), the enrichment (EI), structure index (SI) and channel index (CI) can be calculated. According to Ferris et al., (2001), the following guidelines for specific diagnostics and expected condition of soil food webs in different primary production systems can be used as illustrated in Table 3.2 below:

Table 3.2: Guidelines for specific diagnostics and expected condition of soil food webs (Ferris et al., (2001)

Primary production system	Quadrat (Food web condition)
Annual cropping systems	
Conventional	D (degraded), A (disturbed), B (structured)
Low-input	B (structured)
Organic	A (disturbed), B (structured)
Perennial cropping systems	
Conventional	B (structured), C (stable)
Low-input	C (stable)
Organic	B (structured), C (stable)

A high or low EI describes a nutrient enriched or depleted soil ecosystem by predicting the responsiveness of the guilds to the food source enrichment defined via the enrichment index. According to Habig et al., (2015), low or high SI describes the soil ecosystem regardless of whether the soil ecosystem is disturbed/degraded or structured/matured. According to Ferris et al., (2001), the association of the bacterivores and fungivores is reflected by the CI which is used to signify the bacterial ($CI < 50$) or fungal ($CI > 50$) domination of organic decomposition pathways. Ratios of these indices thus provide valuable information regarding the soil food web structure, enrichment, and decomposition channels. According to Gomes et al., (2003), the level of the soil disturbance can be measured and implemented by assessing the changes in diversity and trophic structure (feeding groups) within a community.

3.3.2 Soil Microbial Diversity

The same three cover crop treatments which were sampled for nematodes, were then again sampled four months later for soil microbial analyses. The reason why the two sample dates differed was because it was important to sample nematodes soon after green manuring so that any biofumigation effect on nematodes as a result of the presence of substances like glucosinolates from the cover crops could be recorded whereas more time was needed to see the impact of the green manuring on soil microbes. This was because the plant material had to first break down and decompose before its effect on soil microbes would have been visible. Four composite (each replicate made up from 6 sub-samples) soil samples were collected from each of the three replicates of the remaining successful three cover crops e.g. (*Lablab purpureus* (Dolichos bean), *Brassica rapa* (Mustard/Caliente) and *Eruca sativa* (Nemat/Rocket) and the control (fallow land/Green Pepper) for soil microbial diversity analyses. The samples were collected three months after green manuring. The soil samples were sent to the laboratory at the Agri Technovation Micro Life Research Centre in Cape Town. Soil analysis of each of the composite samples followed standard laboratory procedures (Habig et al., 2018):

a) Determination of the percentage of soil organic carbon

The determination of the percentage of soil organic carbon was based on wet oxidation by the Walkley-Black method.

b) Determination of soil active carbon

Active carbon was determined as described by Heckman et al., (2009), to measure soil organic matter which is in a readily utilisable form due to the action of soil micro-organisms. The dried soil samples were mixed with 0.02 M $KMnO_4$ solution, shaken, diluted and the colour-change spectrophotometrically measured at 550nm.

c) Determination of soil microbial functional diversity

When carbon sources are utilised, whole-community substrate utilisation profiles (CSUP) are assessed. The collected soil samples were inoculated into Biolog EcoPlates (Trademark of Biolog® Inc., Hayward, USA) containing 31 carbon sources and were diluted in distilled water and a control well, in triplicate (Buyer & Drinkwater, 1997). The Ecoplates were incubated at 28°C. Respiration of carbon sources by microbial populations reduces the tetrazolium dye, causing a colour change which was measured twice daily over a period of 7 days at 590 nm to determine average well-colour development (AWCD) (Winding & Hendriksen, 1997). The amount and equitability of carbon substrates metabolised were used as indicators of richness and evenness to determine the functional diversity of the soil microbial populations (Habig et al., 2018).

d) Determination of soil microbial enzyme activity

Acid phosphatase, ß-glucosidase and urease activities in the soil samples measured to analyse the ability of the soil microbial population to obtain carbon, phosphorus and nitrogen. The calculation of phosphatase activities and ß-glucosidase were determined by the release of p-nitrophenyl after the incubation of soil with p-nitrophenyl phosphate and p-nitrophenyl glucoside, respectively. Urease activity was determined where released ammonia was measured after the incubation of soil samples with a urea solution, where after results were calculated with reference to the calibration curve (Habig et al., 2018).

3.3.3. Data Analysis

STATISTICA 13 data analysis software system (StatSoft Inc. Tulsa, OK, USA) was used to perform the non-parametric statistical analyses of the nematode functional groups, soil microbial diversity indices and enzymatic activity. All the laboratory experiments conducted were repeated on different test dates. Factorial ANOVA were used to determine substantial variances between treatment combinations while the data acquired was analysed by means of a one-way analysis of variance (ANOVA). Homogenous grouping with Fisher's Least Significant Difference (LSD) test was calculated at the 5% level of significance ($p < 0.05$). The NINJA automated calculation web interface was used to demonstrate nematodes as indicator organisms of the nematodes faunal analysis conducted (Sieriebriennikov et al., 2014).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

Six cover crops were initially cultivated for the purpose of the study, although not all these grew successfully to maturity. This was as a result of unforeseen circumstances and conditions beyond control, but the trials were nonetheless completed with the successful cover crops. Criteria for selection of successful cover crops was based on crop performance, i.e. germination, growth and reaching flowering stage successfully with a good crop stand which are general requirements for successful green manuring of cover crops. The analyses of nematodes after a few days after green manuring were used to essentially compare the impact of cover crops (after green manuring) on nematodes. A second part of the study was to compare the impact of the green manured cover crops on aspects of soil microbiology and finally to correlate the analysis for nematodes with the soil microbiology. In hindsight, it would have yielded more and better data if all soil analyses could have been conducted at planting, crop maturity and finally after green manuring, but this could not be helped as these analyses are expensive and the funding available for the study could only afford one soil microbial analyses even when the nematode analysis was sponsored by the ARC.

4.1. Nematode Analysis

There are several functional groups into which nematodes can be divided depending chiefly on their feeding preference: omnivores, predators, bacterivores (bacterial feeders), fungivores (fungal/hyphal feeders), plant feeders, and facultative fungal or plant epidermal cell and root hair feeders (Yeates et al., 1993). For the purpose of this study, population numbers of the bacterivores, fungivores, omnivores and predators were determined as the composition of these nematode communities correlate well with nitrogen cycling and decomposition. This group of nematodes are known as free-living nematodes. In addition, the relative abundance of each of these feeding groups was used as an indicator of environmental change or environmental stress. For example, high population numbers of bacterivores indicate that a good food source is available pointing to a possible source of enrichment. Low numbers of omnivores and predators are indicative of environmental changes more often in agricultural soils due to pesticide use, mono-cropping and mechanical damage due to tillage etc. Plant nematodes collected and identified in this study have previously been reported in South Africa (Kleynhans et al., 1999

4.2. Results from samples taken after green manuring of cover crops

The laboratory number (ARC_PHP #) was allocated to each sample to simplify the labels in the histograms. See table 4.1 below. These laboratory numbers were used in histograms and faunal profiles.

Table 4.1: Samples taken after green manuring of cover crops.

Sample #	Associated Plant	ARC-PHP #
1	*Lablab purpureus* (Dolichos Beans)	D_3.1
2	*Lablab purpureus* (Dolichos Beans)	D_3.2
3	*Lablab purpureus* (Dolichos Beans)	D_3.3
1	*Eruca sativa* (Rocket/Nemat)	N_3.1
2	*Eruca sativa* (Rocket/Nemat)	N_3.2
3	*Eruca sativa* (Rocket/Nemat)	N_3.3
1	*Brassica rapa* (Mustard)	M_3.1
2	*Brassica rapa* (Mustard)	M_3.2
3	*Brassica rapa* (Mustard)	M_3.3

Table 4.2: Plant-parasitic nematodes identified from samples taken after green manuring of cover crops.

Nematodes	Common name	Trophic level
Criconema mutabule	Ring nematode	Ectoparasite
Dorylaimellus sp.	-	Ectoparasite
Helicotylenchus dihystera	Spiral nematode	Ectoparasite
Meloidogyne sp.	Root-knot nematode	Sedentary endoparasite
Pratylenchus brachyurus	Lesion nematode	Migratory endoparasite
Pratylenchus zeae	Lesion nematode	Migratory endoparasite
Rotylenchulus sp.	-	Sedentary endoparasite
Rotylenchus unisexus	Spiral nematode	Semi-endoparasite
Scutellonema brachyurus	Spiral nematode	Semi-endoparasite
Tylenchus sp.	-	Algal, lichen or moss feeders
Xiphinemella sp.	-	Ectoparasite

The soils of this sampling date of all treatments were generally dominated by bacterial feeders and plant feeders (Figure 4.1) while hyphal feeders were fewer in number. The least dominating feeding groups were the predators and the omnivores in most samples, which is also generally significantly lower than the first two sampling dates in this report. The juveniles of *Rotylenchus unisexus* and *Scutellonema brachyurus* are almost identical and could therefore not be separated during the counting process. Therefore, these two species were counted as one population (See Appendix A, Table 1). The same applies for *Pratylenchus brachyurus* and *Pratylenchus zeae*.

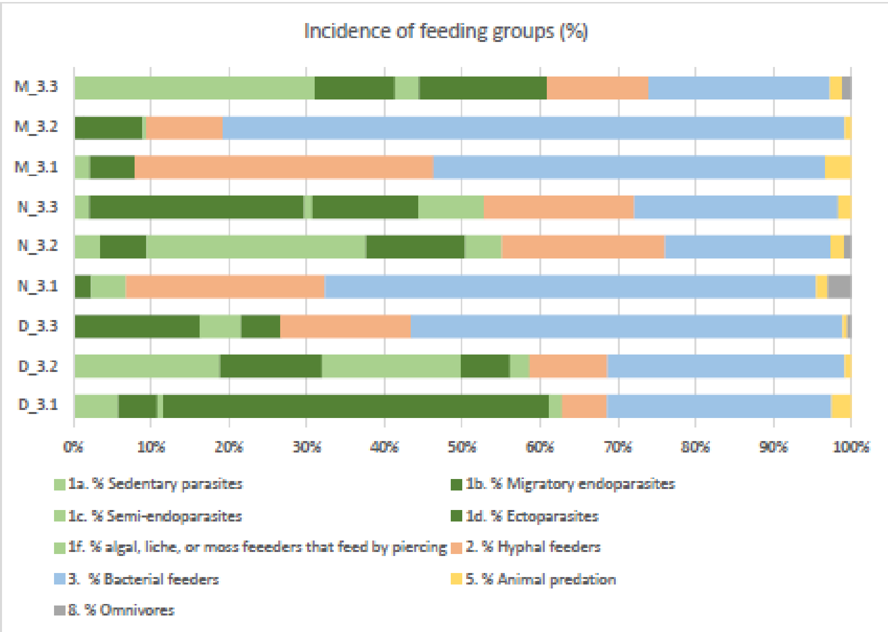

Figure 4.1: Incidence of feeding groups (%) from samples taken after green manuring of cover crops

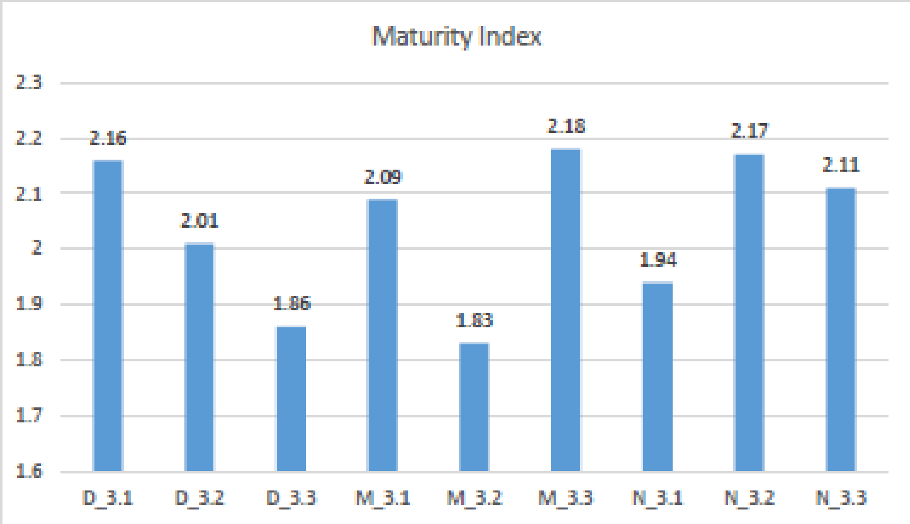

Figure 4.2: Maturity Index from results of sampling taken after green manuring of cover crops

The MI for the sampled soils analyses for this sampling date ranged from 1.83 to 2.18 indicating disturbed soils. None of the sampled soils had an MI value higher than 2.5 indicating all the soils were highly disturbed. This can be understood as the process of green manuring greatly disturbs the soil when the green plant matter is ploughed under.

The free-living nematodes were represented by bacterivores in the cp-class 1-3, fungivores in the cp-class 2-4, omnivores in the cp-class 4 and predators in the cp-class 3-5. In general, cp-class 2 nematodes were dominant in all soils.

Table 4.3: Cp-class for samples taken after green manuring of cover crops

Nematodes	**P-p class**	**C-p class**	**Feeding type**
Criconema mutabile	3	-	Plant feeder - ectoparasites
Dorylaimellus sp.	5	-	Plant feeder - ectoparasites
Helicotylenchus dihystera	3	-	Plant feeder - semi-endoparasites
Meloidogyne sp.	3	-	Plant feeder - sedentary parasites
Pratylenchus brachyurus, Pratylenchus zeae	3	-	Plant feeder - migratory endoparasites
Rotylenchulus sp.	3	-	Plant feeder - sedentary parasites

Rotylenchus unisexus	3	-	Plant feeder - semi-endoparasites
Scutellonema brachyurus	3	-	Plant feeder - semi-endoparasites
Tylenchus sp.	2	-	Plant feeder - algal/lichen/moss feeders
Xiphinemella sp.	4	-	Plant feeder - ectoparasites
Aphelenchoides	-	2	Hyphal feeders
Aphelenchus	-	2	Hyphal feeders
Diphtherophora	-	3	Hyphal feeders
Ditylenchus	-	2	Hyphal feeders
Acrobeles	-	2	Bacterial feeders
Acrobeloides	-	2	Bacterial feeders
Cephalobus	-	2	Bacterial feeders
Cruznema	-	1	Bacterial feeders
Eucephalobus	-	2	Bacterial feeders
Mesorhabditis	-	1	Bacterial feeders
Panagrolaimus	-	1	Bacterial feeders
Plectus	-	2	Bacterial feeders
Prismatolaimus	-	3	Bacterial feeders
Wilsonema	-	2	Bacterial feeders
Discolaimoides	-	5	Animal predation
Discolaimus	-	5	Animal predation
Mylonchulus	-	4	Animal predation
Tripyla	-	3	Animal predation
Aporcelaimellus	-	5	Animal predation
Aporcelaimus	-	5	Animal predation
Dorylaimidae	-	4	Omnivorous

Based on the faunal profile of the samples (Figure 4.3: Faunal profile) the soils sampled are indicative of disturbed and depleted soils. These results confirm the absence of high cp-value nematodes.

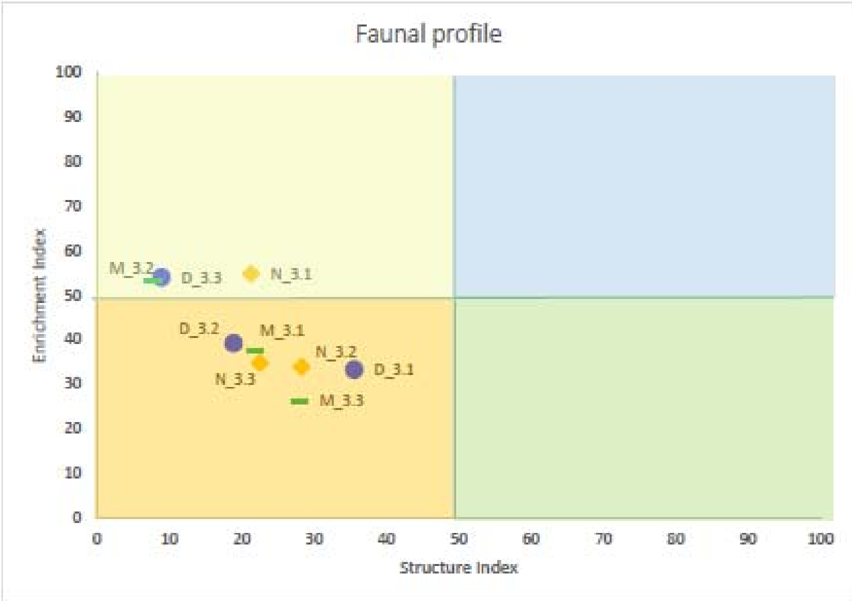

Figure 4.3: Faunal Profile of samples taken after green manuring of cover crops

4.3. Soil Microbial Analysis

According to Grierson et al., (2004), soil microbial activity achieves critical soil biogeochemical processes in soil ecosystems, such as the decomposition of complex molecules into simple monomers that can be easily assimilated by plant roots. In a soil sample, the activity of any enzyme analysed is the sum of active and potentially active enzymes from all the different sources. Microbial activities in the cycling of carbon (ß-glucosidase), nitrogen (urease) or release of inorganic phosphorus (phosphatase) in soil were used to assess soil fertility or to define ecosystem functioning (Habig & Swanepoel, 2015; Utobo & Tewari, 2015).

Twelve soil analyses for soil microbial diversity were done from soil samples taken from three cover crops treatment plots (*Lablab purpureus* (Dolichos bean), *Brassica rapa* (Mustard) and *Eruca sativa* (Nemat)) after green manuring and from the control (Fallow land where the last crop had been green pepper).

Table 4.4: Samples taken after green manuring

CODE	TREATMENT
NR 01	NEMAT
NR 02	NEMAT
NR 03	NEMAT
NR 04	NEMAT
MR 01	Mustard
MR 02	Mustard
MR 03	Mustard
MR 04	Mustard
DR 01	Dolichos
DR 02	Dolichos
DR 03	Dolichos
DR 04	Dolichos
CR 01	Fallow (Green Pepper)

4.3.1. Organic carbon

The percentage soil organic carbon content was determined and represented the percentage organic carbon (total carbon minus the inorganic carbon) stored in soil organic matter. Organic carbon enters the soil ecosystem through decomposition of organic residues, root exudates, living/dead soil organisms mineralised (converted from an organic to an inorganic compound) by soil microbes and consequently utilised as a food and energy source. Results of the percentage soil organic carbon in the respective treatments are presented in Figure 4.4 and Appendix B: Table 4a.

The percentage organic carbon content in the respective treatments are considered high. From the obtained results, the control treatment (Green pepper) displayed the highest percentage soil organic carbon, whereas the Mustard and Nemat treatments displayed a similar low percentage soil organic carbon; the percentage organic carbon in the Mustard treatment was slightly lower compared to the Nemat treatment. The percentage soil organic carbon content in the Dolichos treatment was higher compared to the Mustard and Nemat treatments, but lower than the control. According to the results in Appendix B: Table 4b, the differences in percentage soil organic carbon content between treatments were not statistically significant ($p > 0.05$).

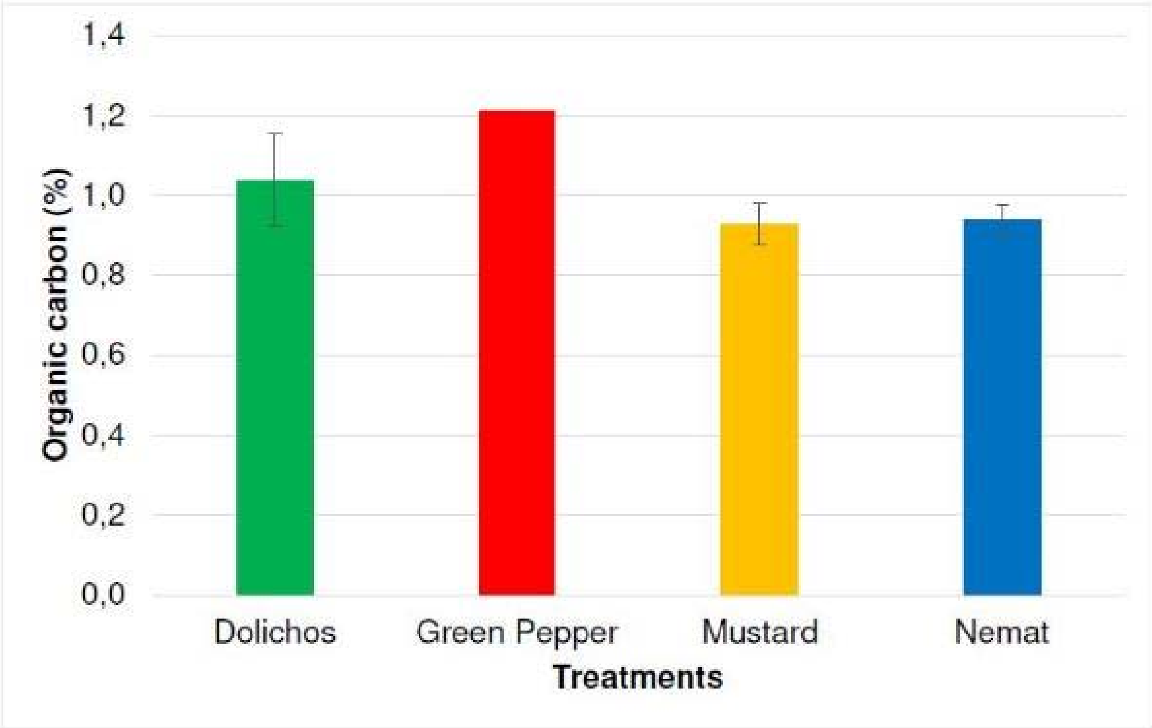

Figure 4.4: Percentage soil organic carbon available in the respective treatments. Treatments with overlapping error bars do not differ statistically significant ($p > 0.05$).

4.3.2. Active carbon

Active carbon is the small fraction of the soil organic matter that is readily available as a carbon and energy source for soil microbes. The results of the active carbon (mg.kg-1) in the respective treatments are presented in Figure 4.5 and Appendix B: Table 5a. These results are good indicators of soils' response to changes in agricultural practices. The active carbon content in the respective treatments varied between intermediate to high. According to the results in Appendix B: Table 5b, the control treatment (Green pepper) displayed a statistically significant higher ($p < 0.05$) active carbon content compared to the Mustard and Nemat treatments which displayed the lowest active carbon content. Differences in active carbon content between the Mustard and Nemat treatments were not statistically significant ($p > 0.05$). The active carbon content in the Dolichos treatment was not significantly lower or higher ($p > 0.05$) compared to the control or Nemat treatments, respectively, but was significantly higher compared to the Mustard treatment ($p < 0.05$).

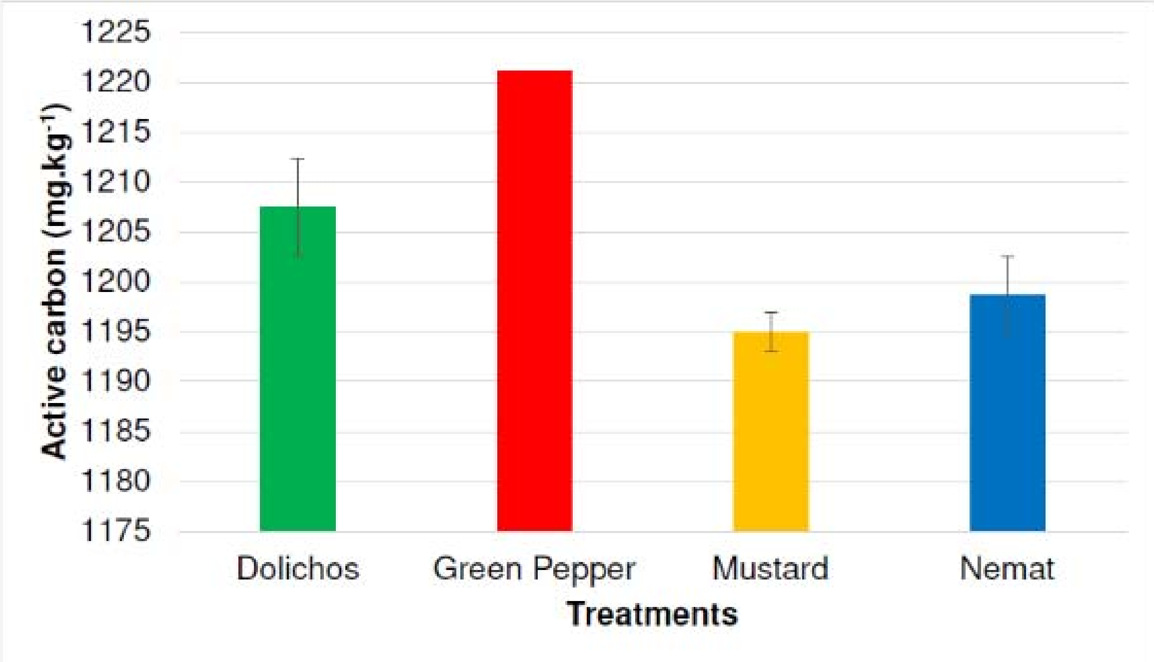

Figure 4.5: Amount of active carbon available in the respective treatments. Treatments with overlapping error bars do not differ statistically significant ($p > 0.05$).

4.3.3. Microbial functional diversity

The mechanism of colour development in Biolog EcoPlatesTM is related to differences in carbon source utilisation (CSU), *i.e.* food source consumption, which, in turn, appears to relate to the diversity of viable microorganisms able to utilise the substrates ("food sources") within the wells of the EcoPlates as a sole carbon source. The diversity of microorganisms correlates strongly with various agricultural factors such as the degree of soil disturbance, the crops planted and the available organic soil cover (*i.e.* compost, mulch, etc.). Microbial diversity therefore provides an indication of the variety of functions that could be performed by soil microbial communities. The higher the diversity, the more microbially-mediated functions could be performed simultaneously to provide in the needs of crops and to improve soil health and fertility.

The Shannon-Weaver substrate diversity index is used to quantify the functional diversity of soil microbial communities based on the amount of different carbon sources utilised by soil microbial communities in Biolog EcoPlatesTM, *i.e.*, comparable to species richness in the soil. Values of the index typically range between 1.5 and 3.5, but rarely increase above 4.5. Depending on the treatments, varying degrees of carbon sources were utilised, with values ranging from 1.80 (intermediate) to 2.77 (high) (Appendix B: Table 6a).

On the other hand, the Evenness index is used as an indication of how abundant species are within a soil microbial community, *i.e.*, how close in abundance the different microbial species are in a soil microbial community. If the abundance of different species in a community is measured, it will invariably be found that some species are rare, whereas others are more abundant / dominant. Substrate evenness assumes a value between 0 and 1, with 1 representing a situation in which all species are equally abundant within the particular microbial population present in the samples. This means less variation in microbial populations between species, thus, less dominance, and higher diversity. Substrate evenness indices obtained for the different sampling sites ranged between 0.54 (low) and 0.85 (high) (Appendix B: Table 6a).

The utilisation of carbon sources by active microbial communities between the different treatments is illustrated by means of a Scatter Chart in Figure 4.6. Apart from the Mustard sample's microbial diversity profile present in the Transition quadrant, the remaining microbial diversity profiles are present in the Ideal quadrant, demonstrating an intermediate to high microbial richness, and an intermediate microbial abundance. The Dolichos treatment demonstrated a significantly higher ($p < 0.05$) microbial richness and abundance compared to the Mustard treatment, whereas the Green Pepper and Nemat treatments did not differ significantly ($p > 0.05$) from each other, or the other treatments (Appendix B: Table 6b). These results depict the CSU of the soil microbial populations present, clearly indicating differences in carbon source utilisation profiles between the different sampling sites, also implying differences in microbial functioning between the different sampling sites. Results demonstrated a lower microbial richness in the Mustard treatment with a higher possibility of dominance by specific microbial species which could be attributed to the known bio fumigation effect of Mustard.

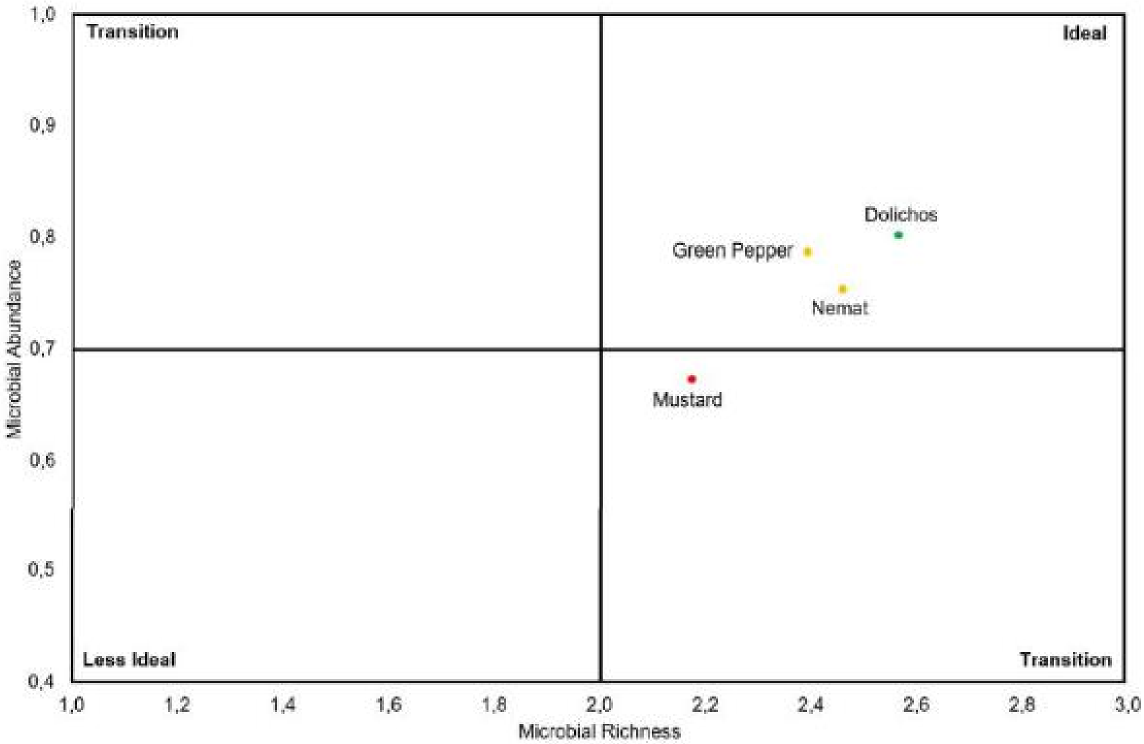

Figure 4.6: Microbial diversity profile representing the average Shannon-Weaver (microbial richness) and Evenness (microbial abundance) indices for the different treatments.

Cluster analysis was performed as an additional measure to enable a 2-D visualisation of the different groups illustrated in Figure 4.7. The resulting dendrogram was constructed with the aid of Cluster Analysis to assign similar treatments into the same clusters as illustrated in Figure 4.7. The soil microbial profiles from the different treatments corroborated the results in Figure 4.7, with similar microbial diversity profiles in the Green Pepper and Nemat treatments (**Green Block**). The microbial diversity profiles in the Green Pepper and Nemat treatments were more closely related/similar to the microbial profile in the Dolichos treatment (**Blue Block**). The microbial diversity profile of the Mustard treatment (**Red Block**) clustered separately from all the other treatments, supporting the hypothesised bio fumigation effect of the Mustard treatment.

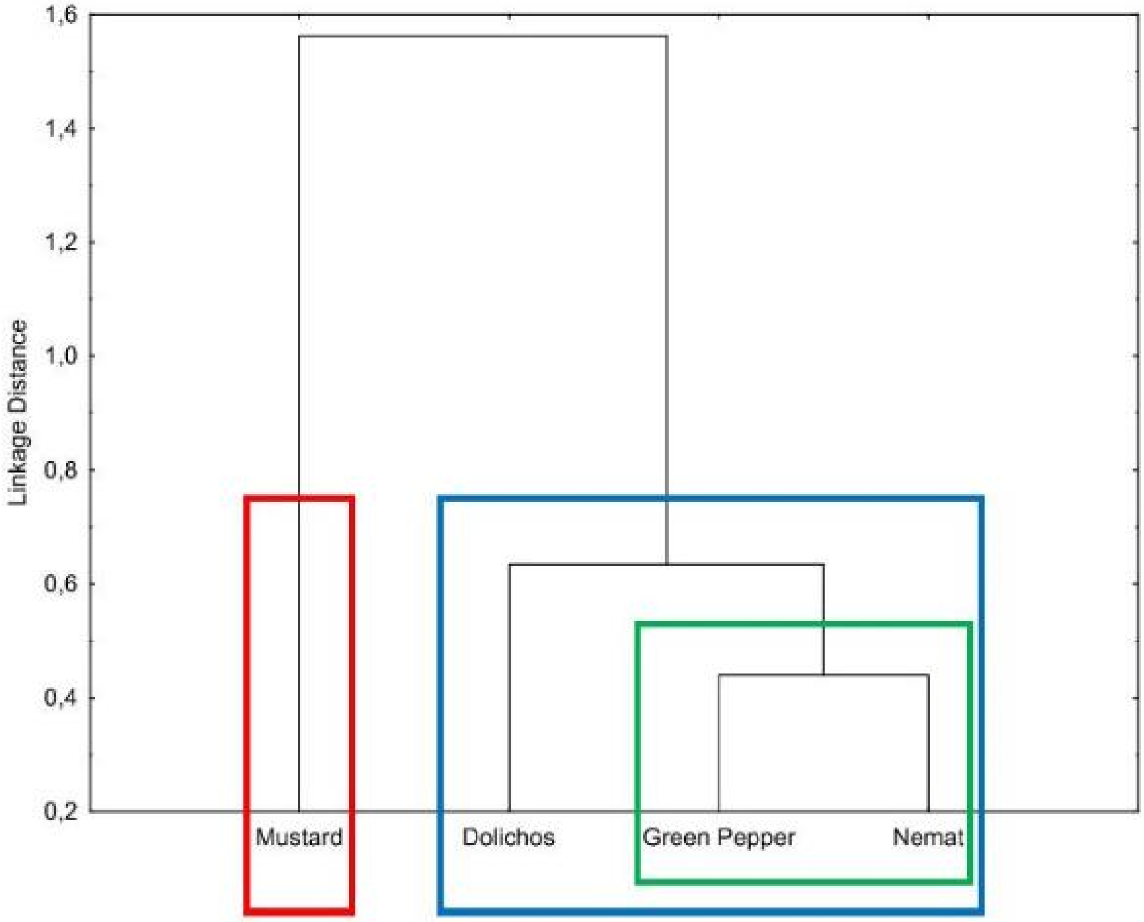

Figure 4.7: Differences in microbial diversity profiles between the various treatments.

4.3.4. Determination of soil microbial enzymatic activity

The activities of four soil microbial enzymes were analysed for this trial: ß-glucosidase (Fig. 4.8), alkaline phosphatase (Fig. 4.9), acid phosphatase (Fig. 4.9) and urease (Fig. 4.10). Results are presented as a means of determining the potential of a soil to degrade or convert (mineralise) substrates from an organic form into plant-available nutrients. By implication: the higher the microbial activity (*i.e.* mineralisation rate), the faster the nutrients that are released from organic substrates will be made available to the soil ecosystem and rhizosphere to be taken up by plant roots.

Soil microbial communities associated with the various treatments differed in their ability/potential to mineralise/convert carbon (ß-glucosidase), phosphorous (acid and alkaline phosphatase), and nitrogen (urease) (Appendix B: Table 7a). Soil microbial communities demonstrated the ability to mineralise phosphorous faster under lower soil pH conditions ($p < 0.05$), compared to under high soil pH conditions ($p > 0.05$) (Fig 4.9). Microbial communities in the various treatments displayed overall intermediate C, P and N mineralisation rates. Considering the overall / combined microbial activity, the microbial communities present in the Green Pepper treatment demonstrated the highest activity compared to the Mustard treatment, which displayed the lowest. According to Appendix B: Table 7b, soil microbial communities in the Green Pepper treatment displayed a significantly higher ($p < 0.05$) carbon mineralisation rate compared to the other treatments (Fig. 4.8), whereas the Dolichos treatment displayed a significantly higher ($p < 0.05$) nitrogen mineralisation rate compared to the Mustard treatment (Fig. 4.10). The Dolichos treatment also demonstrated the highest phosphorous mineralisation rates, irrespective of the soil pH conditions. No statistically significant differences ($p > 0.05$) in alkaline phosphatase activities were observed between treatments, but the Mustard treatment demonstrated significantly lower ($p < 0.05$) acid phosphatase activities compared to the Dolichos treatment (Fig. 4.9).

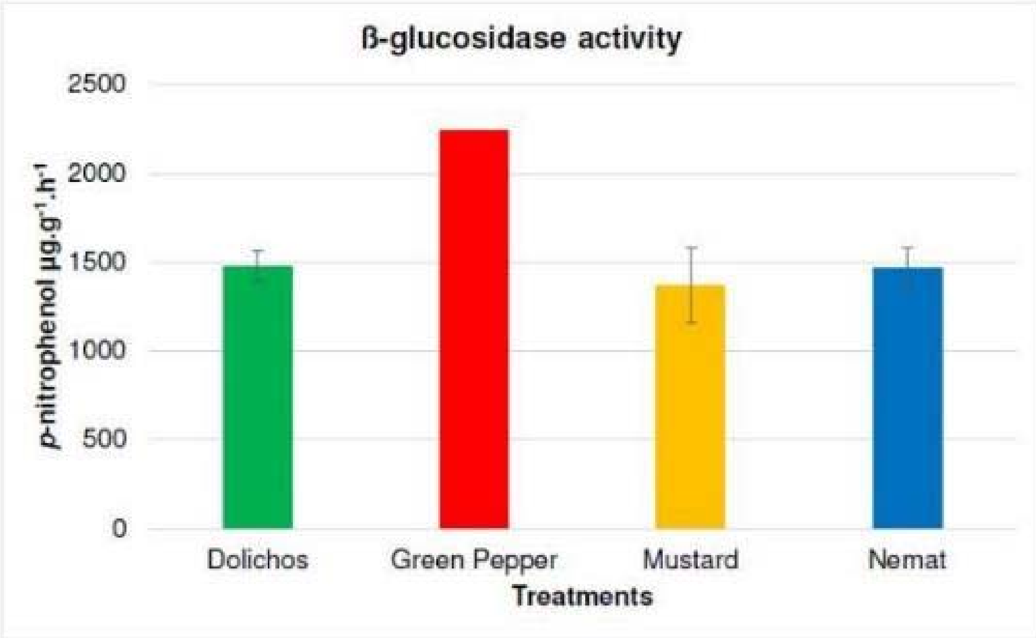

Figure 4.8: Soil microbial ß-glucosidase activity in soils of the various treatments.

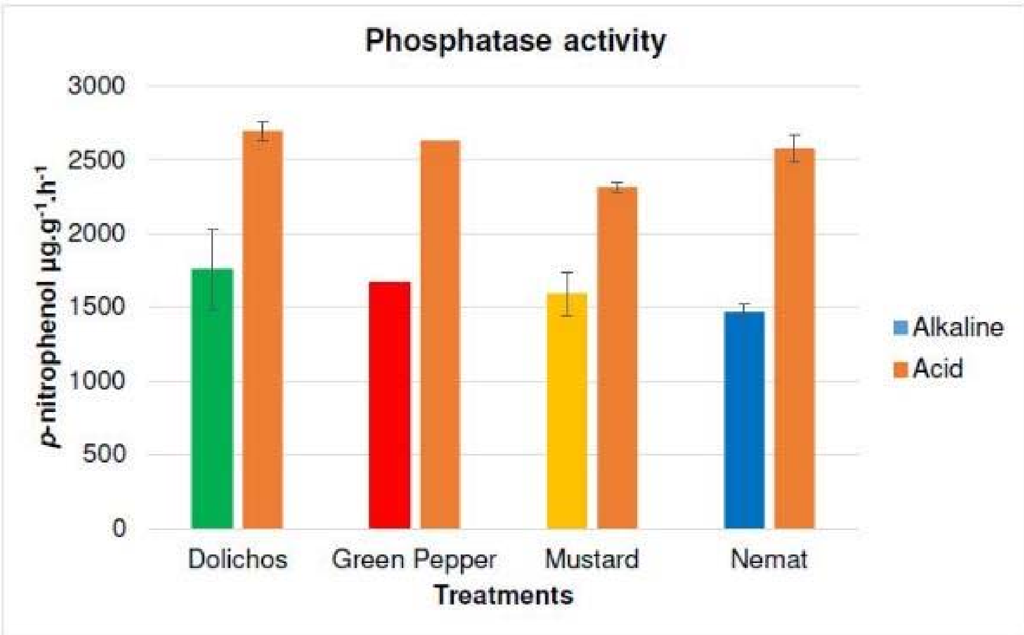

Figure 4.9: Soil microbial alkaline and acid phosphatase activities in soils of the various treatments.

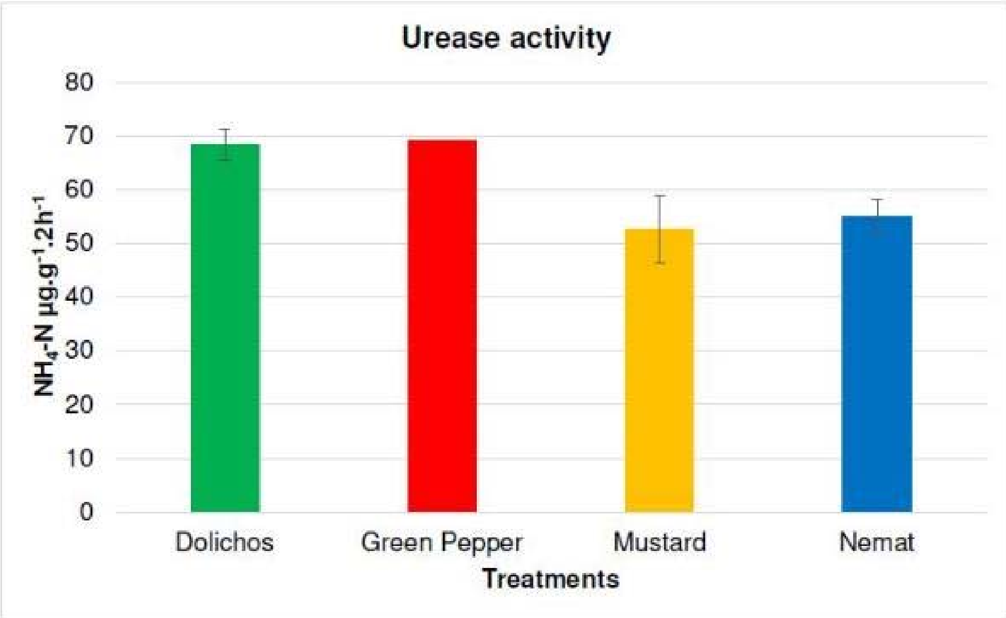

Figure 4.10: Soil microbial urease activity in soils of the various treatments.

Cluster analysis was performed to construct dendograms as an alternative measure to assign similar microbial activities at the various treatments into the same clusters as illustrated in Figure 4.11.

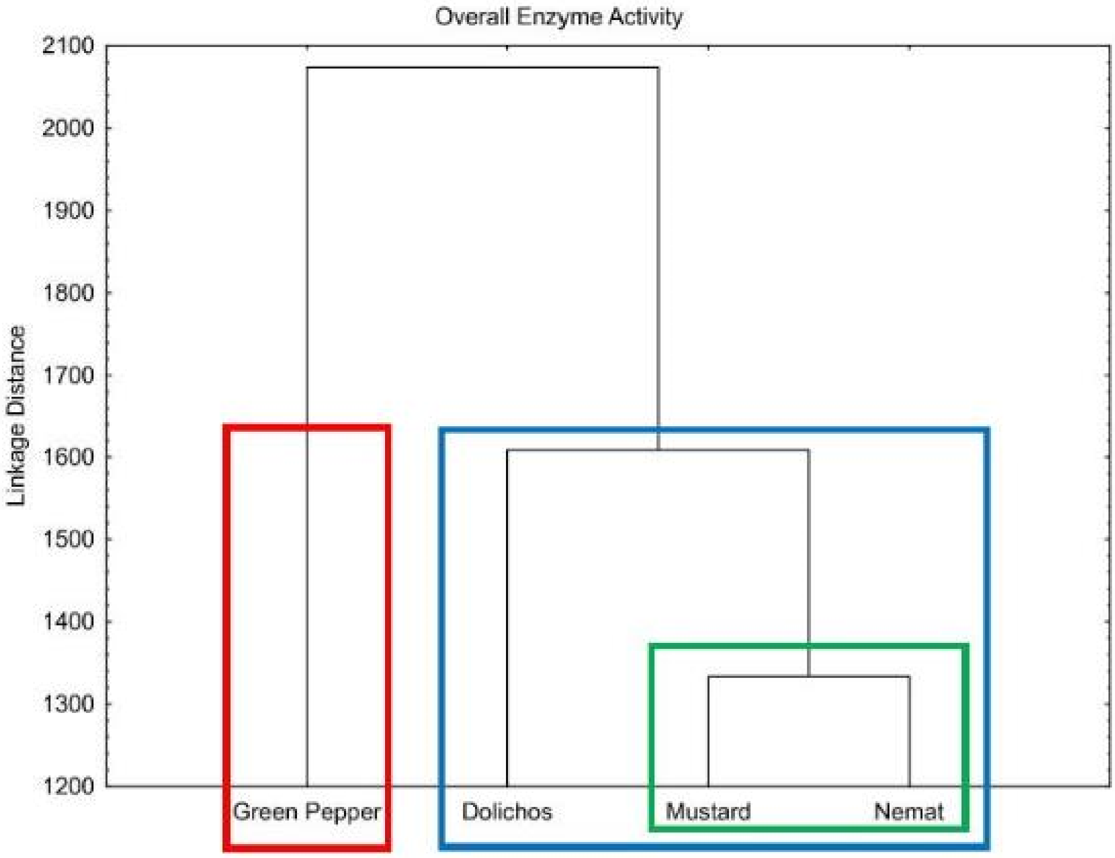

Figure 4.11: Clustering of similar microbial activities at the various sampling sites.

The cluster analyses revealed that the overall microbial activity in the Nemat and Mustard treatments (**Green Block**) were similar, while the microbial activity in the Dolichos treatment (**Blue Block**) was also similar to a lesser degree to the microbial activity in the Nemat and Mustard treatments. The overall microbial activity in the Green Pepper treatment (**Red Block**), clustered separately from all the other treatments. These observations are also supported by the results in Appendix B Table 5(a)(b), indicating that the overall microbial activity in the Green Pepper treatment was the highest compared to all the other treatments, whereas the overall microbial activity in the Nemat and Mustard treatments were the lowest, depending on the specific enzyme activity.

4.4. Integration of nematode and soil microbial analyses

In order to conclude the existence of the association between microbial and nematode (parasitic and non-parasitic) community structures, integration of such data sets was done by subjecting it to Redundancy Analyses (RDA). Principal component analysis was conducted on the soil analyses conducted on the nematodes after green manuring and the soil microbial analyses (also taken from the same treatments after green manuring) to illustrate correlations between the different variables. Results are depicted in Figure 4.12.

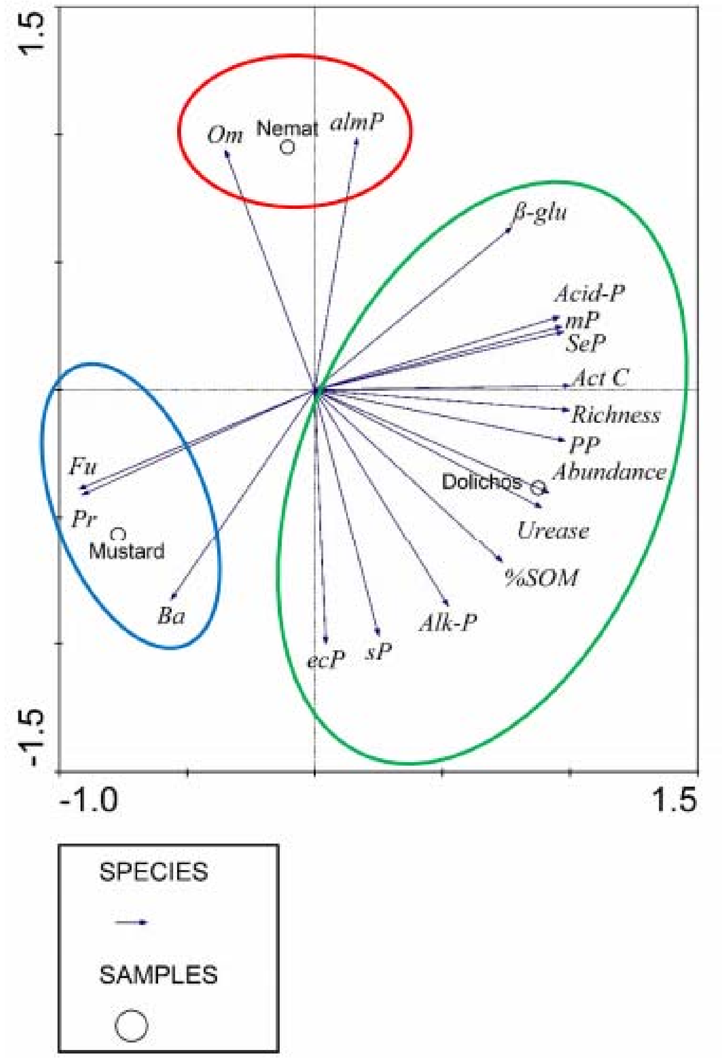

Figure 4.12: Principal Component Analysis (PCA) ordination diagram illustrating inherent differences between the different cover crop treatments with relation to soil microbial diversity and activity characteristics and nematode populations. Microbial activity: ß-glu = ß-glucosidase; Acid-P = acid phosphatase; Alk-P = alkaline phosphatase; Urease = urease; Act C = active carbon; %SOM = % soil organic matter. Microbial diversity: Richness, Abundance. Nematodes: almP = total algal, lichen, moss feeders; mP = total migratory endoparasites; SeP = total semi-endoparasites; PP = total plant-feeding nematodes; sP = total sedentary parasites; ecP = total ectoparasites; Ba = total bacterial feeders; Pr = total animal predators; Fu = total hyphal (fungal) feeders; Om = total omnivorous nematodes.

From the results obtained, each of the different cover crop treatments had its own unique microbial profile, with the Nemat treatment clustering at the top, the Dolichos treatment to the right, and the Mustard treatment to the left of the graph. From Figure 4.12, it is clear that the Nemat treatment showed a positive association with the omnivorous and algal, lichen and moss feeding nematodes, whereas the Mustard treatment showed a positive association with most of the beneficial free-ling nematodes, i.e. the hyphal (fungal) feeders, bacterial feeders and the animal predatory nematodes, and the Dolichos treatment showed a positive association with the majority of the plant-parasitic nematodes and soil microbial diversity and activity.

The negative association of the Mustard treatment with the plant-parasitic nematodes and soil microbial diversity and activity might be attributed to the biofumigation effect of Brassica species, whereas the Dolichos treatment was prone to attack from plant-parasitic nematodes (i.e. sedentary, migratory, ecto- and semi-endoparasitic nematodes). Apart from the strong association with the plant-parasitic nematodes, the Dolichos treatment also associated very strongly with high microbial diversity (Richness, Abundance) and activity. The strong association of the urease and phosphatase activities (alkaline and acid phosphatase) could be attributed to the nitrogen and phosphorous mineralisation capabilities of the microbes generally associated with legumes, or from the high percentage soil organic matter associated with the Dolichos treatment.

The negative association of the % SOM with the Nemat and Mustard treatments could be attributed historical agricultural practices. The distant association of the ß-glucosidase activity with the %SOM might indicate a low C:N ratio organic matter due to the nitrogen contribution from the legume crop. The positive association of the microbial richness and abundance with active carbon, % SOM and plant-parasitic nematodes could imply two scenarios: 1) the increased diversity of soil microbial communities due to increased quantities of root exudates due to root damage caused by the plant-parasitic nematodes, or 2) the presence of the soil organic material and more readily-available primary food source (i.e. carbon) due to the increased active carbon content. Although a strong positive association is expected between the soil microbial diversity and the bacterial-feeding nematodes, the contrary is observed. This indicates a scenario where the more abundant the bacterial-feeding nematodes, the lower the soil microbial diversity.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The aim of this study was to assess the potential of cover crops when green manured, to improve soil conditions with respect to quality and health. This was assessed by determining how the green manured cover crops influenced the nematode communities, and the soil microbial activity in the soil. Although there were differences between the different cover crops as indicated in the previous chapter, cover crops did not overall increase soil microbial activity significantly and nematode community makeup did not significantly change after the cover crop treatments.

Results obtained also indicated that no substantial variances occurred in terms of nematode (parasitic and non-parasitic) community structures and population levels among soils collected from the various cover crops treatments. In terms of non-parasitic nematodes, bacterivores dominated in soils from the three cover crops treatments with individuals of the Ectoparasite and Sedentary endoparasite trophic level being the most abundant. Nematodes belonging to these trophic levels are plant feeders, hyphal feeders and bacterial feeders. Soil samples were mainly dominated by bacterial feeders. This dominance was followed by hyphal feeders and plant feeders. The least dominating feeding groups were the predators and the omnivores in most samples. The latter two trophic nematode groups are considered as K-strategists (persisters) and are the most sensitive to environmental disturbances and stress and the presence of these nematodes also increases the structure of soil food webs.

The maturity index for most of the cover crops treatments in this study were relatively low with values lower than 2.5, which further supports the conclusion that most of the soil were highly disturbed. These low values are mainly due to the absence of sensitive nematode groups (predators and omnivores). Plant-parasitic nematode assemblages (identified from soils of all sampled cover crop treatments) were dominated by the spiral nematodes (belonging to the genera *Helicotylencus* and *Rotylenchus*) followed by *Pratylenchus*, *Tylenchorhynchus*, *Rotylenchulus* and *Meloidogyne*. High plant-parasitic numbers can be challenging in terms of damage that may be inflicted by these pests when such sites are in future considered for the cultivation of crops.

Like nematode community structure results, soil microbial functional diversity and activity were sensitive to, and varied between, the various treatments. According to the results from the three cover crop treatments plus the control that were sampled and analysed, the differences in percentage soil organic carbon content between treatments were not statistically significant ($p > 0.05$). From the obtained results, the control treatment (Fallow land previously planted with Green pepper) displayed the highest percentage soil organic carbon and active carbon content, whereas the Mustard and Nemat treatments displayed equally low percentage soil organic carbon and active carbon content, the percentage organic carbon in the Mustard treatment was slightly lower compared to the Nemat treatment. The percentage soil organic carbon content and active carbon content in the Dolichos treatment was higher compared to the Mustard and Nemat treatments, but lower than the control.

Given the results obtained, the microbiological soil health seems to be more favourable in the Dolichos and Green Pepper treatments because of the high microbial diversity and activity, compared to the soil health status in the Nemat and the Mustard treatments, with the latter demonstrating the lowest soil health status. Consequently, the latter two treatments might hinder crop production directly through lower mineralisation rates, or indirectly through the presence of a low microbial diversity. These observations might have been greatly influenced by the available percentage organic carbon and active carbon which correlated strongly with the overall microbiological soil health.

Since percentage soil organic matter and active carbon are very important sources of food for microbes, the control treatment seems to have already given the microbes a "head start" with the amount of food available. Except for the Control treatment, the Dolichos treatment showed the highest percentage soil organic matter and active carbon content, which naturally benefited the microbes. In final conclusion, the trials revealed some interesting aspects of the effects of green manuring of cover crops on soil heath aspects but were unfortunately not comprehensive enough to make good application recommendations for use in agriculture. More comprehensive studies are therefore needed where these cover crops (and others) are tested more extensively and more soil samples are taken over different phases of the cultivation and green manuring periods which should provide more data which is really needed to provide clear strategies on the use of different cover crops for the improvement of soil conditions as investigated in the study.

5.2. Recommendations

Subsequent to careful consideration of all the results acquired in this study, a few recommendations are made for forthcoming studies:

i) For any future cover crop projects, it is highly recommended that trends in microbial diversity and enzymatic activity be monitored over an extended period in order to attain a more complete indication of the impact different treatments/crops/agricultural practices might have on microbial diversity and activity as an indicator of soil fertility and health. Integration and comparison of available data with more soil quality indicators could contribute to more refined answers and possible predictions.

ii) Should research like this be conducted in future, it should consist of extra sampling periods at some point of the growing season of the cover crops. The motivation for this is that beneficial nematode community structures may change over the season (from planting to green manuring of the cover crops) due to biotic and abiotic factors that affect such organisms. It is desirable that microbe and nematode samples be taken three times per season so that the changes in the populations can be more thoroughly observed. It is strongly recommended that the microbe and nematode analyses be done from the same soil sample.

The recommendations will possibly lead to a better understanding of the effect and impact of cover crops on the environmental and agricultural systems as a whole. It will also provide farmers with more information on the benefits of healthy sustainable soils that contain beneficial organisms.

CHAPTER 6: REFERENCES

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APPENDIX A: TOTAL NEMATODES IDENTIFIED IN SAMPLING

Table 1: Total nematodes identified in sampling results at green manuring of cover crops

Sample #	Associated Plant	ARC-PHP #	Total plant feeding	1a. Total Sedentary parasites	*Meloidogyne* sp. (J)	*Rotylenchulus* sp. (J)	1b. Total Migratory endoparasites	*Pratylenchus zeae* and *Pratylenchus brachyurus*	1c. Total Semi-endoparasites	*Helicotylenchus dihystera*	*Scutellonema brachyurus* and *Rotylenchus unisexus*	1d. Total Ectoparasites	*Criconema mutabile*	*Dorylaimellus* sp.	*Xiphinemella* sp.	1f. Total algal, lichen, or moss feeders that feed by piercing	*Tylenchus* sp.	2. Total Hyphal feeders	*Aphelenchoides*	*Aphelenchus*	*Diphtherophora*	*Ditylenchus*	3. Total Bacterial feeders	*Acrobeles*	*Acrobeloides*	*Cephalobus*	*Cruznema*	*Eucephalobus*	*Mesorhabditis*	*Panagrolaimus*	*Plectus*	*Prismatolaimus*	*Wilsonema*	5. Total Animal predation	*Aporcelaimellus*	*Aporcelaimus*	*Discolaimus*	*Discolaimoides*	*Mylonchulus*	*Tripyla*	8. Total Omnivorous	Subfamily Dorylaiminae
1	Dolichos	D_3.1	380	35	35		30	30	5		5	300	295	5		10	10	35	5			30	175	130	20			5		15		5		15	10	5					0	
2	Dolichos	D_3.2	1430	460	440	20	320	320	435	240	195	155	145	5	5	60	60	245	15	100		130	745	220	340		25	65		55		35	5	20	20						0	
3	Dolichos	D_3.3	525	0			320	320	105		105	100	95	5		0		330	15	100	5	210	1095	425	185	30	210	195		50				10	10						10	10
1	Nemat	N_3.1	45	0			0		0			15	15			30	30	170	30	140			420	60	60		30	195	30	45				10	5				5		20	20
2	Nemat	N_3.2	645	40		40	70	70	330		330	150	130	20		55	55	245	10	60		175	250	25	45	50		100			10	20		20		10			10		10	10
3	Nemat	N_3.3	790	30	30		415	415	15		15	205	205			125	125	290	30	70		190	393	125	70		15	168				15		25	5	10		10			0	
1	Mustard	M_3.1	95	25	25		0		0			70	70			0		465	25	330		110	610	185	35			315	35			40		40	10	15				15	0	
2	Mustard	M_3.2	345	0			45	45	0			285	285			15	15	365	25	305		35	2955	85	65		280	2100	315	65	30	15		30	20		10				0	
3	Mustard	M_3.3	795	405	405		135	135	40	15	25	215	215			0		170		90		80	305	175	80			10			40			20	10			10			15	15

APPENDIX B: SOIL MICROBIAL DIVERSITY ANALYSIS

Table 4a. The % soil organic carbon (%C) available in the respective treatments.

Code	Treatment	%C
NR01	Nemat	0,87
NR02	Nemat	0,90
NR03	Nemat	0,93
NR04	Nemat	1,05
MR01	Mustard	1,06
MR02	Mustard	0,81
MR03	Mustard	0,94
MR04	Mustard	0,90
DR01	Dolichos	1,33
DR02	Dolichos	1,02
DR03	Dolichos	1,04
DR04	Dolichos	0,76
CR01	Green Pepper	1,21

Table 4b. The average % soil organic carbon (%C) available in the respective treatments.

Treatment	%C (average)
Dolichos	1,038 ± 0,117 [a]
Green Pepper	1,210 [a]
Mustard	0,928 ± 0,05 [a]
Nemat	0,938 ± 0,039 [a]

* Values followed by a common letter are not significantly different (p > 0.05).

Table 5a. The amount of active carbon (mg.kg-1) available in the respective treatments.

Code	Treatment	Active Carbon ($mg.kg^{-1}$)
NR 01	Nemat	1192,405
NR 02	Nemat	1194,491
NR 03	Nemat	1209,412
NR 04	Nemat	1198,385
MR 01	Mustard	1194,783
MR 02	Mustard	1189,545
MR 03	Mustard	1198,159
MR 04	Mustard	1197,775
DR 01	Dolichos	1194,575
DR 02	Dolichos	1217,390
DR 03	Dolichos	1206,490
DR 04	Dolichos	1211,835
CR 01	Green Pepper	1221,080

Table 5b. The average amount of active carbon (mg.kg-1) available in the respective treatments.

Treatment	Active Carbon ($mg.kg^{-1}$)
Dolichos	1207,573 ± 4,870 ^{bc}
Green Pepper	1221,080 ^{c}
Mustard	1195,065 ± 1,989 ^{a}
Nemat	1198,673 ± 3,788 ^{ab}

* Values followed by a common letter are not significantly different ($p > 0.05$).

Table 6a. The influence of different treatments on soil microbial species richness (Shannon-Weaver Index) and microbial species abundance (Evenness Index).

Code	Treatment	Shannon-Weaver Index	Evenness Index
NR01	Nemat	2,54	0,80
NR02	Nemat	2,28	0,68
NR03	Nemat	2,64	0,79
NR04	Nemat	2,38	0,74
MR01	Mustard	1,99	0,64
MR02	Mustard	1,80	0,54
MR03	Mustard	2,39	0,73
MR04	Mustard	2,51	0,77
DR01	Dolichos	2,68	0,85
DR02	Dolichos	2,44	0,81
DR03	Dolichos	2,77	0,83
DR04	Dolichos	2,38	0,71
CR01	Green Pepper	2,39	0,79

Table 6b. The influence of different treatments on the average soil microbial species richness (Shannon-Weaver Index) and abundance (Evenness Index).

Treatment	Shannon-Weaver Index	Evenness Index
Dolichos	2,57 ± 0,09 [b]	0,80 ± 0,03 [b]
Green Pepper	2,39 [ab]	0,79 [ab]
Mustard	2,17 ± 0,17 [a]	0,67 ± 0,05 [a]
Nemat	2,46 ± 0,08 [ab]	0,75 ± 0,03 [ab]

* Values followed by a common letter are not significantly different ($p > 0.05$).

Table 7a. The influence of different treatments on enzymatic activities.

Code	Treatment	Activity			
		ß-glucosidase	Alkaline Phosphatase	Acid Phosphatase	Urease
		(*p*-nitrophenol µg.g^{-1}.h^{-1})			(NH_4-N µg.g^{-1}.2h^{-1})
NR 01	Nemat	1249,434	1338,487	2495,899	49,361
NR 02	Nemat	1402,820	1450,414	2371,035	52,216
NR 03	Nemat	1446,730	1567,588	2626,593	54,518
NR 04	Nemat	1770,234	1517,552	2813,368	63,989
MR 01	Mustard	1727,220	1791,648	2299,773	62,026
MR 02	Mustard	956,313	1157,346	2248,426	36,831
MR 03	Mustard	1757,711	1650,586	2416,769	63,088
MR 04	Mustard	1034,446	1744,194	2297,913	48,471
DR 01	Dolichos	1722,445	2469,923	2774,328	69,541
DR 02	Dolichos	1465,967	1888,983	2819,084	74,368
DR 03	Dolichos	1425,034	1311,183	2633,864	68,726
DR 04	Dolichos	1306,572	1341,782	2554,255	60,666
CR 01	Green Pepper	2241,525	1673,167	2629,047	69,049

Table 7b. The influence of different treatments on the average enzymatic activities.

Treatment	Activity			
	ß-glucosidase	Alkaline Phosphatase	Acid Phosphatase	Urease
	(*p*-nitrophenol µg.g^{-1}.h^{-1})			(NH_4-N µg.g^{-1}.2h^{-1})
Dolichos	1480,005 ± 87,595 [a]	1752,968 ± 273,370 [a]	2695,382 ± 61,398 [a]	68,325 ± 2,840 [b]
Green Pepper	2241,525 [b]	1673,167 [a]	2629,047 [ab]	69,049 [ab]
Mustard	1368,922 ± 216,344 [a]	1585,944 ± 145,840 [a]	2315,720 ± 35,720 [b]	52,604 ± 6,222 [a]
Nemat	1467,305 ± 109,474 [a]	1468,510 ± 49,544 [a]	2576,724 ± 94,573 [a]	55,021 ± 3,170 [ab]

* Values followed by a common letter are not significantly different ($p > 0.05$).