INFLUENCE OF PRE-INFECTIONAL AND POST-INFECTIONAL NEMATODE RESISTANCE MECHANISMS IN CROP ROTATION SEQUENCES ON POPULATION DENSITIES OF *MELOIDOGYNE* SPECIES AND SOIL HEALTH

By

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THESIS

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DECLARATION

I, Nyasha Esnath Chiuta, declare that the thesis hereby submitted to the University of Limpopo, for the degree Doctor of Philosophy in Agriculture (Plant Production) has not previously been submitted by me or anybody for a degree at this or any other University. Also, this is my work in design and execution, and all materials contained herein had been duly acknowledged.

Chiuta, N.E (Ms) 08/10/2021

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DEDICATION

I dedicate this work to my beloved parents who are passionate farmers, Mr Jeturo and Mrs Haruzivi Cynolia Chiuta.

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ABSTRACT

Plant-parasitic nematodes inflict economic damages on vegetable and field crops due to a lack of suitable crop protection chemicals and integrated crop management practices. Toxic synthetic chemical nematicides were withdrawn from the agro-chemical markets in 2005 due to their damage to the environment and humans. As such, there is continuous need to develop integrated nematode management strategies that are economic, environment friendly yet capable of effectively controlling the pest to alleviate crop loss and food insecurity. Root-knot (*Meloidogyne* species) nematodes are a major yield- and quality-reducing pest in most potato (*Solanum tuberosum* L.) producing regions in South Africa. However, little is known about the different plant-parasitic nematode species that are associated with potato in some Provinces. The sustainable production of crops in the absence of nematode resistant genotypes depends on the availability of nematode resistant crops in crop rotation systems. However, the effectiveness of these nematode resistance crops in managing root-knot nematodes in potato-based cropping systems has not been investigated in South Africa. The aim of the study was the development of sustainable cropping sequences for management of population densities of *Meloidogyne* species in potato production using crops with different mechanisms of nematode resistance. Two main objectives were investigated, but the second objective was sub-divided into three. The objectives of the study were to investigate (1) whether the diversity and abundance of plant-parasitic nematodes associated with potato in Limpopo Province, would be different to those in other potato-producing regions of South Africa, (2) whether (a) monoculturing potato would have any effects on population densities of *Meloidogyne* species, plant growth and soil health, (b) sequencing potato with a post-infectional nematode resistant crop like *Cucumis africanus* would have any effects on population

densities of *Meloidogyne* species, plant growth and soil health and (c) sequencing potato with a pre-infectional nematode resistant crop such as sweet stem sorghum would have any effects on population densities of *Meloidogyne* species, plant growth and soil health. Ten known nematode genera, namely, *Scutellonema*, *Helicotylenchus*, *Telotylenchus*, *Rotylenchulus*, *Paratylenchus*, *Tylenchorhynchus, Criconema*, *Nanidorus*, *Meloidogyne* and *Pratylenchus* species were present in potato production fields in Limpopo Province, South Africa. The study was conducted on 30 farms, located in Mopani, Sekhukhune, Capricorn, and Waterberg districts by randomly collecting 10 core soil samples per hectare in a zigzag-sampling pattern. A total of eight nematode genera except two (*Meloidogyne* and *Pratylenchus* species) were recorded for the first time in potato fields in Limpopo Province. Additionally, the sampled districts were predominated by different nematode species. The *Meloidogyne* species were the most prevalent nematodes associated with potato crops followed by *Helicotylenchus and Scutellonema* species. In contrast, the *Tylenchorhynchus* and *Nanidorus* species were the least prevalent parasitic nematodes in potato production fields in the Limpopo Province. To achieve Objective 2, two field experiments were conducted at the University of Limpopo (UL) and the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP). In Sequence 1, the treatments (sorghum cv. 'Ndendane', potato cv. ′Mondial G3′, *Cucumis africanus* and potato (cv. ′Mondial G3′)-(Velum) were laid out in a randomised complete block design. In Sequence 2, potato (cv. ′Mondial G3′) was cultivated on all plots as the successor main crop. In Sequence 3, the treatments were laid out as in Sequence 1, whereas in Sequence 4 sole potato crop was cultivated as in Sequence 2. Therefore, four cropping sequences namely, sorghum-potato, potato monoculture, *C. africanus*–potato and potato-(Velum)-potato (control) were investigated simultaneously.

Generally, post-infectional resistant *C. africanus*-potato was more effective than pre-infectional nematode resistant sorghum-potato or potato monoculture cropping sequences in reducing the population densities of *Meloidogyne* species in the soil. This has led to reduced damage to subsequent potato crop providing higher tuber yield, increased shoot mass and nutrients elements accumulation in potato leaf tissues at both sites. The high soil organic carbon content, microbial diversity and enzyme activity observed in *C. africanus*-potato and sorghum-potato showed that these two cropping sequences enhanced soil health better than the monoculture production system of potato with or without Velum application. The different indices (maturity index, channel index, enrichment index and structure index) collectively demonstrated that the soil was highly disturbed with bacteria dominated decomposition pathways. The nematode faunal profile showed that sorghum-potato was the only cropping sequence that improved soil structure as exhibited by high structure index. Therefore, the inclusion of nematode resistant sweet stem sorghum in potato-based cropping system promoted soil health better than the other cropping sequences. In conclusion, *C. africanus*-potato sequence could be used to effectively manage root-knot nematode population densities, whereas sorghum-potato sequence could be considered where the aim is to improve soil health.

ARTICLES GENERATED FROM THE THESIS

1. CHIUTA, N.E., POFU, K.M. and P.W. MASHELA. 2021. Managing thermophilic Meloidogyne species on potato (Solanum tuberosum) under tropical conditions using nematode resistance technologies: A review. *Research on Crops* (Published).
2. CHIUTA, N.E., POFU, K.M. and P.W. MASHELA. 2021. Efficacy of pre- and post-infectional nematode resistant crops in managing *Meloidogyne* population densities in potato-based cropping systems. *Journal of Nematology* (Submitted).
3. CHIUTA, N.E., POFU, K.M. and P.W. MASHELA. 2021. Diversity of plant-parasitic nematodes on potato (*Solanum tuberosum*) in Limpopo Province, South Africa. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science (Submitted).
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5. CHIUTA, N.E., POFU, K.M. and P.W. MASHELA. 2021. Response of potato plant growth and yield to four cropping sequences. *Transylvanian Review* (To be submitted).

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of research problem

Globally, over 100 root-knot (*Meloidogyne* species) nematodes have been identified (Elling, 2013) on over 3 000 host plant species (Belle *et al*., 2019; Moens and Perry, 2009). At least 22% of the identified *Meloidogyne* species occur in Africa in different vegetable and field crops (Belle *et al*., 2019; Karuri *et al*., 2017; Onkendi, 2013), with 7% being associated with potato (*Solanum tuberosum* L.) in South Africa (Onkendi, 2013). The damaging effects of root-knot nematodes have been significant on agricultural crops since the withdrawal of synthetic nematicides from the agrochemical markets (Fourie *et al*., 2017; Silva *et al*., 2013). In crops such as potato that have genotypes which are not resistant to *Meloidogyne* species (Jones, 2006; Macharia *et al*., 2019; Pofu and Mashela, 2017a), tuber yield can be reduced by as high as 50%, with incidences of complete crop failure (Lima *et al*., 2018; Pofu *et al*., 2012). The sustainable production of crops without nematode resistant genotypes depends much on the availability of nematode resistance in crops that could be included in crop rotation systems. Apparently, most resistant crops fail to suppress nematode population densities in crop rotation systems especially when the subsequent crops are susceptible to nematode parasitism (McSorley, 2011; Pofu and Mashela, 2017b).

1.1.2 Impact of research problem

Potato crops could be under great economic damage if the nematode population densities of the different species probably occurring within the fields were above the damage threshold values. Conversely, the failure of nematode resistant crops to suppress nematodes when included in cropping rotation with nematode susceptible crops such as potato could result in great yield and quality losses of the main crop due to damage by the carry-over nematode population densities.

1.1.3 Possible causes of research problem

The acute shortage of nematology expertise in Africa has caused slow progress in nematology research (Coyne *et al*., 2018). For example, most resource-constrained farmers are unaware of the nematode assemblages occurring within their fields or the damage associated with these nematodes, which previously resulted in complete crop failure (Mashela *et al.,* 2017a). Mashela and Pofu (2016) suggested that the mechanism of nematode resistance could play a role in the use of nematode resistance in crop rotation systems. Generally, plants have two mechanisms of nematode resistance, namely, pre-infectional and post-infectional nematode resistance (Huang, 1985; Ramatsitsi, 2018). In pre-infectional nematode resistance, plants develop root structures or produce nematicidal or repulsive root exudates that prevent the entry of second-stage juveniles (J2) into the root system (Jatala and Russel, 1972). In most cases, this mechanism is believed to not benefit the susceptible successor crop since nematodes could enter survival stages when subjected to various environmental factors (McSorley, 2003). In contrast, J2 can penetrate the root system of post-infectional nematode resistant plants, but once inside, the plants produce plant genes that are not compatible with the gene products produced by J2

(Mashela *et al*., 2017a), thereby preventing the establishment of feeding sites (Huang, 1985; Mashela *et al*., 2017a). The effectiveness of nematode-avoidance and resistance mechanisms in nematode suppression in the context of potato-based cropping sequences had not been documented.

1.1.4 Possible solutions to research problem

Plant-parasitic nematode surveys could provide the much-needed information about the different nematodes associated with potato in regions where such information is not well-documented in South Africa. On the other hand, crop rotation systems that include pre- and post-infectional nematode resistant crops would provide relevant information on the efficacy of the two mechanisms in nematode suppression under crop rotation systems. As such, the current study intended to determine the efficacy of pre- and post-infectional nematode resistant plants in sequential crop arrangements that include potato as the main crop, along with their effects on soil health.

1.1.5 General focus of the study

The inclusion of nematode resistant plants to manage plant-parasitic nematodes under field conditions is not new (Navarrete *et al*., 2016). The current study was intended to compare the effectiveness of pre- and post-infectional nematode-resistance mechanisms exhibited in locally adapted crops in managing population densities of *Meloidogyne* species in potato-based cropping system and to determine their overall effect on tuber yield and soil health.

1.2 Problem statement

Information regarding the diversity and abundance of plant-parasitic nematodes associated with potato in Limpopo Province is not well-documented (Marais *et al*., 2015). Furthermore, the phasing out of synthetic chemical nematicides exacerbated the challenges of plant-parasitic nematodes in crops with no nematode resistant genotypes (Fourie *et al*., 2017; Silva *et al*., 2013). Basically, there are inconsistent results when potato crops follow nematode resistant crops in crop rotation systems. Sometimes the nematode resistant crops effectively suppress nematode population densities, but at times the system failed to effectively manage targeted nematode population densities (McSorely, 2011; Pofu and Mashela, 2017b). Such observations have been linked to the mechanism of nematode resistance exhibited by the crops used in sequential cropping systems.

Crops such as sweet stem sorghum [*Sorghum bicolor* (L.) Moench], which exhibited pre-infectional nematode resistance against *M. incognita* and *M. javanica* (Mashela and Pofu, 2016), resulted in high nematode numbers on the successor potato crop in a crop rotation system (Pofu and Mashela, 2017b). In contrast, the efficacy of plants with post-infectional nematode resistance such as wild watermelon (*Cucumis africanus* L.F.), which is highly resistant to root-knot nematodes (Pofu *et al*., 2012; Ramatsitsi, 2018) had not been documented in sequential cropping systems where potato is the main crop. Additionally, the influence of including pre- and post-infectional nematode resistance crops in potato-based cropping sequence on soil health has not been documented.

1.3 Rationale of the study

Globally, potato is one of the four major stable agronomic crops, producing more than 350 million metric tons per annum, worth approximately US$140.5 billion on 19 million ha arable land (Devaux *et al*., 2020; FAOSTAT, 2018). In South Africa, potato is the most important field crop (Potato SA, 2020). The identification of plant-parasitic nematode diversity associated with potato is a necessary precursor to the successful implementation of any nematode management strategy (Onkendi and Moleleki, 2013; Marais *et al*., 2015). For example, when potato cyst (*Globodera rostochiensis* Woll) nematode was detected in cooler potato-producing regions of South Africa for the first time, their spread to other areas was successfully managed by implementing strict quarantine measures (Knoetze, 2014; Knoetze *et al*., 2006). Historically, fumigant nematicides were used to manage nematode population densities, with claims that root-knot nematodes were not an economically important pest. However, after the withdrawal of fumigant nematicides from the agrochemical markets, it was shown that there were no potato genotypes that were resistant to *Meloidogyne* species. Hence, nematode resistant crops could be included in potato-based cropping systems to reduce plant-parasitic nematode damage.

The mechanism of nematode resistance could elucidate why some crops are not effective in suppressing nematode population densities in crop sequences. Generally, nematodes can enter chemiobiosis, which is cryptobiosis in response to chemicals (Mashela and Pofu, 2016), and this could explain why synthetic chemical nematicides failed to eliminate most plant nematodes. Similarly, certain crops, particularly those with pre-infectional nematode resistance, could also fail to suppress nematode population densities that could detrimentally affect the successor crop in sequential

cropping systems. Cryptobiosis is a process whereby nematodes enter a state of reduced respiration to the minimum after being gradually exposed to unfavourable conditions, making certain stages of nematodes to be highly tolerant to the adverse conditions (Mashela, 2007). Cryptobiosis was reported for water stress (anhydrobiosis), oxygen deficiency (anoxybiosis), low soil temperature (cryobiosis), chemicals (chemiobiosis) and salinity (osmobiosis) (Mashela, 2007). In context of crop rotations, pre-infectional nematode resistant plants which release chemicals such as root exudates into the rhizosphere could potentially encourage cryptobiosis, with the surviving nematode stage serving as a resource for attacking the successor crop. On the contrary, in crops with post-infectional nematode resistance, cryptobiosis would hardly occur since J2 are allowed to penetrate the roots, with plant genes being activated to trap nematodes inside the roots (Mashela *et al*., 2017b).

Generally, when one practice is introduced to solve one challenge, certain unpredicted challenges emerge. For example, when highly effective fumigant nematicides were introduced after World War 2 (Taylor, 2003), the products were biocidal (Abawi and Widmer, 2000), resulting in a wide array of emerging challenges, which included the deterioration of soil health (Meena *et al*., 2020). As such, environment-friendly nematode management alternatives such as the inclusion of nematode resistant crops had to be developed. Certain crops, such as cotton (*Gossypium hirsutum* L.), with large taproot systems, are known to have positive effects on soil health (Ma *et al*., 2013; Paez-Garcia *et al*., 2015). Consequently, the choice of crops for inclusion in cropping sequences should be supported by empirical evidence that they would not have detrimental effects on soil health.

1.4 Purpose of the study

1.4.1 Aim

The development of sustainable cropping sequences for the management of *Meloidogyne* species population densities in potato production using crops with different mechanisms of nematode resistance.

1.4.2 Objectives

The objectives were grouped into two main objectives, with the second objective having three sub-objectives. Therefore, the objectives of the study were to:

1. Determine whether the diversity and abundance of plant-parasitic nematodes associated with potato in Limpopo Province, would be different to those in other potato-producing regions of South Africa.
2. The three sub-objectives were to investigate whether:

   (a) monoculturing potato would have any effects on population densities of *Meloidogyne* species, plant growth and soil health,

   (b) sequencing potato with a post-infectional nematode resistant crop like *C. africanus* would have any effects on population densities of *Meloidogyne* species, plant growth and soil health, and

   (c) sequencing potato with a pre-infectional nematode resistant crop like sweet stem sorghum would have any effects on population densities of *Meloidogyne* species, plant growth and soil health.

1.4.3 Null hypotheses

1. The diversity and abundance of plant-parasitic nematodes associated with potato in Limpopo Province, would not be different to those in other potato-producing regions of South Africa.
2. Three null hypotheses from the sub-objectives:

   (a) monoculturing of potato would not have any effects on population densities of *Meloidogyne* species, plant growth and soil health,

   (b) sequencing potato with a pre-infectional nematode resistant crop like sweet stem sorghum would not have any effects on population densities of *Meloidogyne* species, plant growth and soil health and,

   (c) sequencing potato with a post-infectional nematode resistant crop like *C. africanus* would not have any effects on population densities of *Meloidogyne* species, plant growth and soil health.

1.5 Reliability, validity and objectivity

Reliability was ensured by using appropriate levels of statistical significance ($P \leq 0.05$). Validity was achieved by repeating the same experiment at different locations (Little and Hills, 1981). Objectivity was achieved by discussing the findings based on empirical evidence as shown by statistical analyses, with findings compared against results from other studies in order to eliminate all forms of subjectivity (Little and Hills, 1981).

1.6 Bias

Bias was minimised by reducing the experimental error in each experiment through (a) adequate replications, (b) blocking for the heterogeneous distribution of soil nematode population densities, (c) assigning the treatments at random within the selected research design and (d) proper plot techniques where non-treatment factors were maintained uniformly for all experimental units (Gomez and Gomez, 1984; Little and Hills, 1981).

1.7 Scientific significance of the study

Findings of the current study intended to identify plant-parasitic nematodes that were associated with potato in Limpopo Province, along with suitable cropping systems intended for managing nematode population densities in potato production systems. Outcomes of the current study were also intended to provide information on potential capabilities of pre- and post-infectional nematode resistance crops in managing nematode population densities of *Meloidogyne* species when included in sequential crop arrangements, along with their impact on soil health, which could translate to sustainable agro-systems.

1.8 Structure of the thesis

The thesis comprised five chapters, with Chapter 1 providing a detailed description of the research problem. Chapter 2 contained a literature review, which focused on the work done while identifying knowledge gaps on the research problem. The research Chapter 3 addressed Objective 1, whereas research Chapter 4 addressed sequentially, the three components of Objective 2. In Chapter 5, findings in all chapters were summarised and integrated to provide the significance of the study, followed by

recommendations for future research. The Harvard author-alphabet format was adopted for all the in-text citations and final reference list as prescribed by the relevant University of Limpopo-approved policy framework. Data used in the research Chapters were made available in analytical form in a list of Appendices, whereas raw data were deposited in the University data bank for storage. In the next chapter, the researcher reviewed literature on the work done while identifying knowledge gaps on the research problem.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Potato (*Solanum tuberosum* L.) is one of the most important field crops in the Republic of South Africa (Onkendi and Moleleki, 2013), occupying from 50 000 to 54 000 ha of arable land (Potato SA, 2017). Potato production occurs in 16 regions in South Africa. However, 70% of the annual total potato production occurs in three provinces, namely, Limpopo, Western Cape and Free State (Potato SA, 2017). Globally, plant-parasitic nematodes are a major yield- and quality-reducing pest in potato-producing regions (Lima *et al*., 2018; Onkendi and Moleleki, 2013; Van der Waals *et al*., 2013). Prior to the withdrawal of the highly effective fumigant nematicides from the agrochemical markets in 2005, root-knot (*Meloidogyne* species) nematodes were considered a minor pest in potato production. However, after the withdrawal, it was realised that this nematode genus was a major pest and that none of the potato cultivars had genotypes which were resistant to *Meloidogyne* species (Pofu and Mashela, 2017a).

Various nematode management strategies have been employed previously (Bello, 1998). Prior to the withdrawal of fumigant nematicides in 2005, most farmers were used toxic synthetic chemicals. Hence, the re-introduction of effective, environment-friendly strategies became the focal research niche. As such, the objective of this chapter was to review work done while identifying knowledge gaps on nematode distribution in potato fields and the potential use of nematode-resistance mechanisms in management of root-knot nematodes population densities.

2.2 Work done on the problem statement

2.2.1 Plant-parasitic nematodes affecting potato production in South Africa

Globally, root-knot nematodes are among the top three important nematode pests that affect potato production, following the two cyst nematodes (*Globodera* species), *G*. *rostochiensis* and *G*. *pallida* Stone (Grabau and Noling, 2019). In the United States of America, *M. chitwoodi* and *M. hapla* can cause great economic damage on potato crops more than any other *Meloidogyne* species (Ingham *et al*., 1999). The southern root-knot nematode, *M. incognita*, was mainly identified in most potato-producing regions within the tropics and subtropics (Onkendi and Moleleki, 2013). The genera mostly associated with potato in South Africa in decreasing order of importance include *Meloidogyne* species, root-lesion (*Pratylenchus* species), potato-cyst (*Globodera* species) and stubby root (*Nanidorus* species) nematodes (Jones *et al*., 2017; Kleynhans, 1978). Generally, *Heterodera* species are the most economically important plant-parasitic nematodes on potatoes in temperate regions (Lima *et al*., 2018), with limited distribution in warm regions. However, recent evidence suggested that the potato cyst (*Globodera rostochiensis* Woll) nematode is increasingly becoming widely distributed within the tropical areas in Africa (Coyne *et al*., 2018). In South Africa, *G. rostochiensis* is the least prevalent since it is mostly confined within the few cold potato-producing areas in the Western Cape Province (Knoetze, 2014). Generally, 95 plant-parasitic nematode species were previously associated with potato in South Africa (Marais *et al*., 2015). However, this could be an underestimation since non-georeferenced data were excluded from the cited surveys. In addition, acute shortage of nematology experts in certain provinces limited survey progress in plant-parasitic nematodes, especially in Limpopo Province, where most smallholder farmers cannot pay for nematode survey services. Climate change, such as the predicted rise

in temperature might increase occurrence and distribution of thermophilic plant-parasitic nematodes (Mashela *et al*., 2017b; Van der Waals *et al*., 2013). Potato crops do well in sandy soils, which are unfortunately the most preferred by *Meloidogyne* species (Engelbrecht, 2012). Also, recent studies suggested that a certain very aggressive root-knot nematode, *M. enterolobii* Yang and Eisenback, with a life cycle of less than 15 days, is increasingly becoming widely distributed in Limpopo Province (Collett, 2020; Maleka, 2021). As such, congruent to identifying the exact *Meloidogyne* species in potato-producing regions, nematode management strategies that limit nematode damage should be empirically developed.

2.2.2 Economic damage of root-knot nematodes on potato

Worldwide, plant- parasitic nematodes can cause an annual crop yield loss from 10 to 15%, which translates into a loss of US$ 78 billion (Lima *et al*., 2018). Root-knot nematodes contribute greatly to this estimated crop loss since they are polyphagous (Daneel *et al*., 2017; Lima *et al*., 2018). In potato fields that are severely infested with root-knot nematodes, yield loss can be as high as 50% to complete crop failure (Lima *et al*., 2018; Pofu *et al*., 2012). In Australia, Hay *et al*. (2016) suggested that it was difficult to accurately determine yield loss due to root-knot nematode damages since several other pests and diseases, along with abiotic factors, jointly affect potato crops under field conditions. Conversely, tuber yield loss due to root-knot nematode damage is underestimated in Africa due to the unavailability of competent practising plant nematologists (Coyne *et al*., 2018).

Several studies have demonstrated that root-knot nematodes can render the potato tubers unsuitable for fresh market or processing by causing shape deformations, blistering or necrotic spots on tuber surface and reduced tuber size (Ingham *et al*., 1999; Jones *et al*., 2017; Lima *et al*., 2018; Vovlas *et al*., 2005). In some cases, plant growth variables are reduced, resulting in low tuber set and overall poor yield (Patel *et al*., 2020; Vovlas *et al*., 2005). The severity of root-knot nematode damage on potato depends on several factors such as nematode species, prevailing climate conditions in the production region, edaphic factors, cultivar, and initial population densities in the soil (Medina *et al*., 2017; Wesemael *et al*., 2014). Generally, potato monocropping is associated with many adverse effects with regards to root-knot nematode damage since all commercially available varieties are susceptible. To a large extent, monocropping a susceptible crop promotes accumulation of host specific plant-parasitic nematodes (Gowen, 2003; Strom *et al*., 2019). For example, soil nematode population densities of *Meloidogyne* species increased under potato monoculture (Crow *et al*., 2000; Pofu and Mashela, 2017b).

In some cases, plant growth and yield were not influenced by high soil nematode population densities under potato monoculture (Crow *et al*., 2000). For example, soil nematode population densities increased during the first few years (≤5 years), but fluctuated or declined under prolonged monocropping (Zhu *et al*., 2013). The causes for such contradictions were not unpacked. However, the enhancement of soil suppressive effects, for example, promotion of natural enemies of plant-parasitic nematodes, could be the cause for such observations (Eberlein *et al*., 2016). In other cases, monocropping a susceptible crop had no effect on the soil nematode population densities or yield during the first two to four years (Eberlein *et al*., 2016; Zawislak *et*

al., 1981). All this shows the complexity of nematode - host interaction under natural conditions.

2.2.3 Nematode management using post-infectional nematode resistant crops

Post-infectional nematode-resistance mechanism: Ramatsitsi (2018) reviewed a wide range of crops with post-infectional nematode resistance, which included African horned cucumber (*Cucumis metuliferus* L.), cotton (*Gossypium hirsutum* L.), coffee (*Coffea arabica* L.), soybean (*Glycine max* L.), grape vine (*Vitis vinifera* L.), tobacco (*Nicotiana tabacum* L.), carrot (*Daucus carota* L.), chilli pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.) and cowpea (*Vigna unguiculata* L.). Post-infectional nematode-resistance mechanism is triggered when second-stage juveniles (J2) penetrate the root system of the plant (Bent, 1996). The mechanism is associated with hypersensitive response which is clearly explained by the gene-for-gene model (Mashela *et al*., 2017a). In this model, the plant-parasitic nematode gene products are recognised by the resistant gene products of the host trap-crop. The attempt to establish a feeding site or the migration of the nematode in the root causes the production of host gene products. Consequently, cascading effects of biochemical reactions begins to occur (Huang, 1985; Mashela *et al*., 2017a). The activation of physiological and molecular processes results in inhibition of giant cell formation during the sedentary process (Huang, 1985; Mashela *et al*., 2017a).

In some cases, post-infectional resistant crops produce chemicals known as phytoalexins which cause withering of the cells surrounding the nematode (Harborne, 1999). Consequently, the J2 are prevented from proceeding to the site where giant cells would be formed to support feeding (Otipa *et al*., 2003). Eventually, these

nematodes die due to starvation (Navarrete *et al*., 2016). Dhandaydham *et al*. (2008) observed dead undeveloped J2 trapped around withered cells of post-infectional nematode resistant barrel medic (*Medicago truncatula* L.) roots. Correspondingly, root-knot nematodes failed to establish feeding sites on post-infectional resistance grape vine rootstocks and marigold (*Tagetes* species) cultivars (Anwar and McKenry, 2002; Wang *et al*., 2007). Failure of J2 to establish feeding sites promotes conversion of most female J2 to males, which do not require feeding (Fassuliotis, 1970). High maleness index has been associated with plants within the *Cucumis* species exhibiting post-infectional nematode-resistance mechanism (Pofu and Mashela, 2011). Subsequently, egg production becomes low due to reduced adult female nematode population densities (Faske, 2013). High maleness index and low egg population densities were reported on the root system of post-infectional nematode resistant coffee cultivars (Silva *et al*., 2013).

On the other hand, some phytoalexins are highly toxic or nematicidal (Harborne, 1999). For example, marigold, coffee and cotton cultivars released toxic alpha-terthienyl, phenolic compounds and terpernoid aldehydes, respectively which killed plant-parasitic nematodes trapped within the root systems (Silva *et al*., 2013; Veech, 1979; Wang *et al*., 2007).

Hypersensitive response can activate the biochemical defence mechanism, which results in root tip necrosis (Silva *et al*., 2013). Thus, the root tip becomes a physical barrier which prevents further entry of J2 into the root system of the plant. Such defence mechanism was observed on oat (*Avena sativa* L.), grape vine and wild

watermelon (*Cucumis africanus* L.F.) plants grown in root-knot nematode infested soil (Anwar and McKenry, 2002; Marini *et al*., 2016; Mashela, 2002; Ramatsitsi, 2018).

According to Williamson and Hussey (1996), activation of hypersensitive response in crops exhibiting post-infectional nematode-resistance mechanism can be very complicated. The reason for this observation is that the nematode - host interaction is greatly influenced by plant genes and gene products (Mashela *et al*., 2017a). Additionally, other biotic and abiotic factors also play a role in the modulation of the nematode-host interaction (Mashela and Nthangeni, 2002; Pofu *et al*., 2011; Silva *et al*., 2013). In some cases, the nematode-resistance mechanism responses of the plants are overpowered by high initial nematode population densities (Mashela *et al*., 2017a). Additionally, high salinity conditions (Mashela and Nthangeni, 2002) and whitefly attack (Pofu *et al*., 2011) managed to break post-infectional nematode-resistance mechanism in *C. africanus*, resulting in the successful establishment of giant cells by root-knot nematodes. Under salinity conditions, delay or failure of the defence mechanism of a post-infectional resistant plant occurs because of osmoticum ions and osmotic organic compounds that are imbalanced within the nematode-infested plant (Mashela *et al*., 2017a). In some cases, hypersensitive response is delayed or weakened when abiotic or biotic stress factors cause upregulation of plant genes that collaborate with gene products to promote nematode feeding sites formation and secretions of biochemical compounds that protect nematode bodies while inside the root system (Mashela *et al*., 2017a).

The effect of* post-infectional resistant plants *on plant-parasitic population densities: Post-infectional resistant plants can protect themselves from plant-parasitic nematode damage. More importantly, they can be used as dead-end trap crops as a way of managing plant-parasitic nematodes in different cropping systems. For instance, root-knot nematode resistant bahiagrass (*Paspalum notatum* Flugge), wheat (*Triticum aestivum* L.) and tomato cultivars managed to reduce the carry-over root-knot nematode population densities when included in different crop rotation systems, where the successor crops were highly susceptible to nematode damage (Talavera *et al*., 2009; Williamson *et al*., 2013*;* Wright *et al*., 2015). In some cases, the resistant crops were used as pre-plant trap cover crops (Navarrete *et al*., 2016).

Root-knot nematodes are relatively difficult to manage through crop rotation, cover cropping or sequential cropping unlike other plant-parasitic nematode species due to their wide host range (Karpouzas *et al*., 2004). Nonetheless, some antagonistic trap crops have been successfully used to manage root-knot nematodes in different vegetable fields, including potato fields (Mandal and Hossain, 2017; Otipa *et al*., 2003). *Solanum sisymbriifolium* Lam., a post-infectional nematode resistant trap crop reduced potato cyst nematodes by 99% (Dandurand *et al*., 2019). Additionally, post-infectional nematode resistant marigold cultivars were successfully used to manage root-lesion (*Pratylenchus* species) and potato cyst (*Globodera* species) nematodes in potato-based cropping systems (Kimpinski *et al*., 2000; Hooks *et al*., 2010). The effective management of root-lesion nematodes resulted in a tuber yield increase by 14% (Kimpinski *et al*., 2000).

The effect of* post-infectional resistant plants *on yield and morpho-physiological traits of the successor crop: Davis *et al*. (2003) reported a decrease in reniform (*Rotylenchulus reniformis* Linford and Oliveira) nematode population densities and an increase in cotton yield following post-infectional resistant soybean cultivar. Conversely, Lopez-Lima *et al*. (2013) observed a decrease in potato-cyst nematode population densities when post infectional garden pea (*Pisum sativum* L.) and broad bean (*Vicia faba* L.) were sown in potato-based cropping sequences. However, the successful nematode management by these crops had no significant effect on the growth and yield of the subsequent main crop. Similar observations were made when potato followed post-infectional resistant cotton plant in fields predominately infested with *M. incognita* (Crow *et al*., 2000). In other studies, population densities of root-knot nematodes were inversely proportional to yield and growth of the main crop (Abbasi and Hisamuddin, 2014; Korayem *et al*., 2012; Tahery *et al*., 2011). On the other hand, post-infectional nematode resistant tomato plants failed to suppress *M. incognita* soil population densities over three years. However, it is important to note that the effectiveness of a resistant crop depends on several factors such as the plant itself, initial nematode population densities, edaphic factors, and the prevailing temperature. Miller *et al*. (2006) reported that single year rotations using nematode resistant crops were not adequate for nematode management. In cases where hypersensitive reaction is delayed, the effectiveness of poor-host, post-infectional resistant trap-crops can be compromised (Mashela *et al*., 2017a; Silva *et al*., 2013).

2.2.4 Nematode management using pre-infectional nematode resistant crops

Pre-infectional nematode-resistance mechanism: Ramatsitsi (2018) reviewed a wide range of crops with pre-infectional nematode resistance, which included marigold, sorghum (*Sorghum bicolor* (L.) Moench), cabbage (*Brassica oleracea* L.),

asparagus (*Asparagus officinalis* L.), rhodes grass (*Chloris gayana* L.), garlic (*Allium sativum* L.), tomato, velvet bean (*Mucuna pruriens* L.) and strawberry (*Fragaria ananassa* L.). In the current study, nematode resistant sweet stem sorghum cultivar, which was included in the cropping sequences, has pre-infectional nematode resistance. In a previous study, the sweet stem sorghum cv. ′Ndendane -X1‛ prevented *M. incognita* and *M. javanica* J2 from penetrating the root system (Mashela and Pofu, 2016). Similar observations have been reported on other sorghum cultivars which were later used to manage root-knot nematodes in different cropping systems (De Brida *et al*., 2017; McSorley *et al*., 1994; McSorley and Gallaher, 1997). Generally, host plants produce attractive chemical cues such as flavonoids, which serve as cues to the chemosensory receptors of root-knot nematodes for penetration to occur (Chin *et al*., 2018; Huang, 1985; Sikder and Vestergård, 2019). However, in pre-infectional nematode resistant plants, some plants might prevent nematode infection by failing to produce suitable chemical cues (Chin *et al*., 2018). Results attesting to this postulation were reported in a study by Silva *et al*. (2013), where the authors observed that *M. exigua* J2 could not penetrate the root system of coffee cv. ′Apoata‛ plant because the nematodes could not recognise any chemical cues from the roots.

Pre-infectional nematode-resistance mechanism also occurs when plants exude pre-formed root metabolites that repel or reduce mobility of plant-parasitic nematode within the rhizosphere (Huang, 1985; Sikder and Vestergård, 2019). Root exudates of crops such as tomato (Kirwa *et al*., 2018; Yang *et al*., 2016), marigold (Wang *et al*., 2018), Castor (*Ricinus communis* L.) (Dong *et al*., 2018), alfalfa (*Medicago sativa L)* (Postnikova *et al*., 2015), sorghum-sudangrass [*Sorghum bicolor* (L.) Moench × *S. sudanense* (Piper) Stapf)] (Dover *et al*., 2004), successfully repelled J2 of root-knot

nematodes. However, some of the nematode resistant sorghum [*Sorghum bicolor* (L.) Moench] cultivars that successfully suppressed nematode population densities under controlled conditions failed to effectively suppress nematodes under field conditions (McSorley, 2011). Besides repelling the J2, root exudates of some pre-infectional resistant plants impeded the hatching of J2, thereby protecting the plant from nematode infection (Hooks *et al*., 2010).

In certain instances, some pre-infectional resistant plants have root morphological structures capable of preventing J2 penetration and infection (Huang, 1985). The cell walls of roots could be heavily suberised and lignified, thus providing a barrier that prevents nematode penetration (Mashela *et al.*, 2017a; Underwood, 2012). Such structural barriers are the main form of resistance in pepper (*Capsicum annuum* L.) and cotton (Anwar *et al*., 1994; Pegard *et al*., 2005). Holbein *et al*. (2016) suggested that the structural barriers could be ineffective against root-knot nematode infection penetration since after penetration at the elongation zone, J2 move to the root cap, and then upwards through the vascular bundle, thereby avoiding the lignified cells (Mitkowski and Abawi, 2003). Certain biotic stress factors in the rhizosphere have been reported as lignification triggers on cells that would otherwise be non-lignified under normal conditions (Barros *et al*., 2015).

Some pre-infectional nematode resistant plants can exude allelochemicals with nematicidal properties within the rhizosphere. Such a mechanism of resistance had been observed in sorghum-sudan grass, marigold and *Allium* species which produce dhurrin ($C_{14}H_{17}NO_7$), alpha-terthienyl ($C_{12}H_8S_3$) and dimethyl disulphide ($C_2H_6S_2$),

respectively (Haroutunian, 2013; Wang *et al*., 2007; Xie *et al*., 2016). Dhurrin is a glycoside which breaks down to produce hydrogen cyanide (HCN), a highly toxic chemical compound to nematodes (Meyer and Fry, 1978; Mweke *et al*., 2008). Additionally, most sorghum varieties produce sorgoleone ($C_{22}H_{29}O_4$), a root exudate which is also highly toxic to nematodes (Czarnota *et al*., 2003). Sorgoleone is a soil-active hydrophobic compound, with herbicidal properties and can supresses the growth of a wide range of plant species (Dayan *et al*., 2009). The effectiveness of sorgoleone on suppression of population densities of different plant-parasitic nematodes is well-documented (Aissani *et al*., 2015; Andrade *et al*., 2010; Rodriguez-Kabana *et al*., 1992; Wang *et al*., 2002; Xie *et al*., 2016). Sorgoleone-producing sorghum cultivars have the potential to suppress nematode population densities when included in different cropping systems.

The effect of pre-infectional nematode resistant plants on plant-parasitic nematode population densities: The use of nematode resistance crops such as sorghum and sweetcorn in okra (*Abelmoschus esculentus* L.)-based cropping systems resulted in suppression of root-knot nematode population densities by 21% and 44%, respectively (Mweke *et al*., 2008). Similarly, non-host sorghum cultivars reduced root-knot nematode population densities in maize-based (McSorley and Gallaher, 1993), peanut-based (Rodriguez-Kabana *et al*., 1988) and soybean based (Rodriguez-Kabana *et al*., 1991) cropping systems. However, in some cases, the inclusion of pre-infectional nematode resistant plants in nematode management cropping sequences or rotations only benefited the non-host plants and not the susceptible successor crop as intended in such nematode management strategies (Kratochvil *et al*., 2004). Such observations were mostly reported when the pre-infectional resistant plants included

in a cropping sequence did not produce attractive chemicals needed for host recognition by J2, when the non-host plant exuded repulsive chemicals and when the root structure prevented nematode infection penetration (Kratochvil *et al*., 2004). Under such conditions, the various stages of plant-parasitic nematodes enter cryptobiosis until a susceptible host plant which releases suitable chemical cues is cultivated (Hooks *et a*l., 2010; McSorley, 2003). However, sometimes the transitioning of J2 from an active state to a survival state is delayed, thereby causing the lipid reserves to diminish (Hooks *et al*., 2010). Consequently, the J2 become ineffectual in infecting the successor crop (Silva *et al*., 2013). Such observations were observed when marigolds and Italian ryegrass (*Lolium multiflorum* Lam.) were included in a cropping sequence (Hooks *et al*., 2010).

Certain *Brassicaceae* crops, sorghum-sudangrass [*Sorghum bicolor* (L.) Moench × *S. arundinaceum* (Desv.) Stapf] hybrid and velvet bean cultivars exhibited pre-infectional resistance to *Meloidogyne* species under greenhouse conditions, but significantly failed to reduce *M. incognita* population densities under field conditions (Crow *et al*., 2001; Daneel *et al*., 2017). The presence of other *Meloidogyne* species or races and/or other plant-parasitic nematodes in the field, could have reduced the effectiveness of the test non-host crops in managing the test nematode (Crow *et al*., 2001). Apparently, it is important to empirically identify factors which render greenhouse-tested resistant crops ineffective when included in different cropping systems with the intention of managing plant-parasitic nematodes under field conditions. Some studies showed that allelochemicals released by pre-infectional resistant crops were only effective during active growing stages of the effector plant (Cheng and Cheng, 2015; Wang *et al*., 2007). Once the crops were removed, the allelochemicals were quickly mineralised

into nontoxic chemical compounds. However, in some sorghum cultivars, allelochemical residues were broken down into nematicidal components (Mweke *et al*., 2008).

The effect of pre-infectional nematode resistant plants on yield and morpho-physiological traits of the successor crop*:* The successful management of root-knot nematode population densities by non-host sorghum and sweetcorn cultivars resulted in an increase in okra yield by 60 and 92%, respectively (Mweke *et al*., 2008). Similarly, potato tuber increased following non-host pearl millet or marigold cultivars (Belair *et al*., 2005; Kimpinski, 2000; LaMondia, 2006). On the contrary, potato tuber yield was low and plant-parasitic nematode population densities were high following the cultivation of non-host sorghum-sudangrass (LaMondia, 2006). The above contradictory findings suggest that perhaps, other factors other than the targeted plant-parasitic nematodes might have compromised the yield. For example, marigold and sorghum cultivars effectively repelled root-knot nematodes, but simultaneously promoted cyst and lesion nematode infections, respectively, which could threaten growth and yield of the successor crop (Dover *et al*., 2004; Wang *et al*., 2018). Generally, information on the effect of preceding non-host crops on potato growth or tuber yield in root-knot nematode infested fields is very scarce.

2.2.5 Soil health

Soil health is the capacity of the soil to sustain biological productivity, human health, plant and animal productivity and health within natural or man-made ecosystems (Larkin, 2015). Soil is a vital living substance, and its health is influenced by chemical, physical and biological processes that are interrelated (Biswas and Naher, 2019; Collange *et al*., 2011). Chemical properties of soil are due to chemical processes or

reactions that occur in the soil such as pH, electrical conductivity, nutrient status and salinity (Doran and Parkin, 1996). The application of physical force on the soil causes processes and reactions to occur resulting in soil physical properties. The indicators of soil physics provide information about the interaction of soil particles with water, gases, root system and any other substance (Doran and Parkin, 1996). The examples of soil physical properties include infiltration, bulk densities, aggregate stability and soil structure (Doran and Parkin, 1996). Soil biological properties give information about the microbial and faunal activity that form the soil food web and shows their involvement in different process such as decomposition, mineralisation and immobilisation (Doran and Parkin, 1996).

Research interests in soil health studies are currently increasing (Hubanks *et al*., 2018) Although different soil health indicators have been identified, the soil health standards against which soil health can be measured accurately are still unclear (Norris and Congreves, 2018). Soil health studies that have been previously conducted mainly focused on soil chemical indicators followed by biological and then physical indicators (Norris and Congreves, 2018). Soil physical properties were the least studied because soil takes longer to respond to physical-forming factors. According to Doran and Parkin (1996), ideal soil health indicators should be sensitive to natural and human impact, integrate soil physical, chemical and biological attributes, correlate well with vital processes in the ecosystem, should be interpretable and accessible to many users.

Soil biological indicators such as enzyme activity, microbial and nematode communities are excellent indicators of soil health since they respond rapidly to soil

changes (Habig *et al.,* 2018, Li *et al*., 2016; Yeates *et al*., 1993). However, studies showing the use of these indicators to determine soil health status under different vegetable cropping sequences, especially where non-or-poor host crops are used to manage nematode population densities are limited (Norris and Congreves, 2018). In this study, four soil health indicators namely, organic carbon content, microbial diversity, enzyme activity and nematode communities were reviewed.

Soil health indicators

Soil organic carbon content (SOC) is a quantifiable carbon component of soil organic matter (Griffin *et al*., 2013; Singh *et al*., 2012). The main sources of SOC are the decomposed soil fauna and flora, root exudates, plant and animal residues (Edward and Edwards, 2020). The presence of SOC influences the different chemical, physical and biological processes in the soil (Wu *et al*., 2019). Generally, SOC is the main source of food for most soil microorganisms (Wu *et al*., 2019). Therefore, its availability greatly influences microbial community diversity, activity and population densities (Habig *et al*., 2018). The analysis of microbial community functional diversity is based on sole-carbon substrate utilisation using the Biolog® system from which metabolic fingerprints are determined and carbon source utilisation profiles (CSUP) are made (Habig *et al*., 2018, Mofokeng *et al*., 2020). Generally, agricultural soils with high levels of microbial community diversity and activity are desirable under sustainable agricultural practice (Hu *et al.*, 2011).

Soil micro-organisms normally produce and release enzymes which catalyse the decomposition and nutrient cycling processes (Alves *et al*., 2014). Such enzymes are

substrate-specific and can be used to assess the degrading capacity of microorganisms occurring in each soil sample. Over the years, ß-glucosidase, urease and alkalin-phosphatase enzymes responsible for carbon, nitrogen and phosphorus cycling, respectively, have proven to be useful soil health indicators in most agricultural ecosystems (Habig *et al.*, 2018).

Nematode communities have been used in ecological studies as great bioindicators for soil health since they are represented at every trophic level of the soil food web (Ferris *et al.*, 2001; Ferris and Bongers, 2006; Malherbe and Marais, 2015; Ney *et al.*, 2019, Yeates *et al*., 1993). The most common methods of analysing soil nematode communities are through assessing the functional guilds, feeding habits and nematode indices (Bongers and Bongers, 1998; Ney *et al*., 2019, Yeates *et al*., 1993). Functional guilds are groups of nematode species that have similar feeding habits within an ecosystem (Bongers and Bongers, 1998). Feeding habits refer to the different diets of the nematodes within a soil food web (Yeates *et al*., 1993). For example, nematodes that feed on plants, bacteria, fungi, other invertebrates or a combination of diets are known as herbivores, bacterivores, fungivores, predators and omnivores, respectively (Hooks *et al*., 2010, Yeates *et al*., 1993).

Nematode indices provide information about the enrichment, structure and maturity of the ecosystem (Bongers, 1990; Bongers and Ferris 1999; Sieriebriennikov *et al*., 2014). The indices are calculated using the different trophic groups and coloniser-persister (c-p) values (Neher *et al*., 2004; Ney *et al*., 2019; Sieriebriennikov *et al*., 2014). These indices together with general ecological indices such as Shannon-

Weaver and Simpson's index have been used to determine diversity and evenness of species within the nematode assemblages, respectively (Neher and Darby, 2009). Nematode diversity refers to the variety of species that occur within an ecosystem, whereas evenness is the measure of the species relative abundance (Neher and Darby, 2006).

Soil health in different cropping systems

Studies investigating the effect of different nematode management strategies on soil health are limited (Navarrete *et al*., 2016). In this review, non-potato-based cropping systems were also considered since research focusing on potato-based cropping systems is not well-documented.

Generally, the inclusion of different crops in a cropping sequence increases the variety of food source for soil microorganism to feed on (Larkin *et al*., 2010). Thus, in fields where crop rotation or a different crop species are sown in a sequence great soil microbial diversity is observed unlike where monocropping is practiced (Galazka and Grzadziel, 2018; Ingham, 2016). In some studies, nematode population densities, SOC and tuber yield were greatly influenced by sequence, duration and plant species included in potato-based cropping systems (Larkin *et al*., 2010)

Intensive agriculture is practiced in commercial potato production, and this causes high soil disturbances. Such disturbances negatively affect microbial communities in the soil resulting in lower structural and maturity indices compared to pristine environments which have little to no disturbance (Gupta *et al*., 2019; Neher, 2001;

Neher and Darby, 2006). Generally, nematodes in the high trophic levels such as predators are very sensitive to minimal soil disturbance (Hoorman, 2011).

Hooks *et al*. (2010) observed that different marigold cultivars had different effects on beneficial microorganisms. For example*, T. erecta* enhanced bacterial feeders when used to control plant-parasitic nematodes, whereas *T. patula* showed no effect. In another study, marigold cultivars successfully suppressed *M. incognita*, but had no significant effect on free living nematodes (Marahatta *et al*., 2010). In an experiment comparing wheat monoculture and wheat-lupin bean (*Lupinus albus* L.) cropping sequence, free-living nematode diversity was higher in the latter than in the former (Rahman *et al*., 2007).

In another experiment, pre-infectional nematode resistant Sunn Hemp (*Crotalaria juncea* L.) crop improved soil enrichment, but failed to improve soil structure (Hinds *et al*., 2013; Marahatta *et al*., 2010). Therefore, this goes on to show that many factors can affect changes in soil health under natural field conditions (Karlen *et al*., 2019). According to N'Dayegamiye *et al*. (2017), cropping systems where non host plants are included to manage nematodes while promoting soil health and productivity of the main crop are difficult to design.

2.3 Identified knowledge gaps on the problem statement

An updated checklist of plant-parasitic nematodes associated with potato crops in South Africa was last compiled five years ago (Marais *et al*., 2015). However, this

survey did not represent some of the major potato-producing regions such as Limpopo Province. As such, studies investigating the plant-parasitic nematode diversity occurring in potato fields in Limpopo Province are paramount. The determination of predominant nematode species would assist researchers in developing effective nematode management strategies which will reduce nematode damage, tuber loss and improve food security.

In South Africa, all commercial potato cultivars are good host of *Meloidogyne* species (Pofu and Mashela, 2017a). The inclusion of nematode suppressive crops in potato-based cropping systems can be an effective way of managing plant-parasitic nematode damage. Previously, the aforementioned nematode management strategy was not an option because it was associated with reduced profit (Karpouzas *et al*., 2004). However, the banning of toxic synthetic chemical nematicides which were once habitually used to manage root-knot nematode resulted in the need for re-emphasis of environment friendly nematode management strategies. In South Africa, few root-knot nematodes suppressive crops have been identified since these nematodes are highly polyphagous (Daneel *et al*., 2017).

According to Navarrete *et al*. (2016), the efficacy of the nematode suppressive crops on the successor crop is barely evaluated in most studies. Generally, the host-status of the plants used to manage plant-parasitic nematodes in different cropping systems is known. However, the mechanisms of resistance exhibited by these plants are usually not mentioned. Hence, there are little to no information about the most effective nematode resistant mechanism in managing plant-parasitic nematodes in different

cropping sequences. Furthermore, fewer studies assess the effect of including nematode suppressive crops in a cropping sequence on soil health. Currently, studies investigating the effectiveness of pre- and post-infectional nematode resistant crops in potato-based cropping sequences have not been documented in South Africa. In the following research chapter, a survey was conducted to investigate plant-parasitic nematodes associated with potato in Limpopo Province, South Africa.

CHAPTER 3

DIVERSITY OF PLANT NEMATODES ON POTATO IN LIMPOPO PROVINCE, SOUTH AFRICA

3.1 Introduction

Approximately 54 000 ha are being used for the annual production of potato (*Solanum tuberosum* L.) in South Africa (Potato SA, 2017). Limpopo is one of the four major provinces where intensive potato cultivation is practiced (Potato SA, 2017). Following the withdrawal of highly effective fumigant nematicides from the agrochemical markets in 2005, the root-knot (*Meloidogyne* species) nematodes had been one of the major production constraints in various crops (Lima *et al*., 2018; Mashela *et al.,* 2017b; Van der Waals *et al*., 2013). High nematode population densities, when not properly managed, can immensely reduce potato tuber yield and quality. Therefore, potato fields should be regularly surveyed to ensure that nematode population densities are managed to below their respective economic threshold levels using different strategies, which include the use of disease-free seeds and crop rotation systems.

Unfortunately, most smallholder potato farmers can not afford disease-free certified seeds and complex crop rotation systems (Mashela *et al*., 2017b). As such, they risk encountering nematode damage problems in their fields. The high infection risk in conjunction with the limited knowledge on nematode pest parasitism and pathogenicity among most smallholder farmers and some agricultural extension officers who normally advice farmers further intensify the nematode damage problem (Mashela *et al*., 2017b). Therefore, there is need for interactive, practical nematode pest awareness workshops where farmers and extension officers could be trained on the

damaging effects of the pest and sustainable management practices. Already, the South African government took the initiative to enhance food security and rural development by establishing policies that promote need-driven research, resource allocation and income distribution to be fairly directed towards smallholder farmers with the intention of promoting them to be commercial farmers (Mashela *et al*., 2017b).

Prior to the withdrawal of fumigant nematicides South African potato farmers hardly believed that *Meloidogyne* species were a challenge in potato production (Mashela *et al*., 2017b). Following the withdrawal of most products, screening studies in South Africa demonstrated that all available commercial potato cultivars were hosts to *Meloidogyne* species (Pofu and Mashela, 2017a). Additionally, most plant-parasitic nematode surveys associated with potato crop were conducted after the withdrawal of methyl bromide in 2005 (Engelbrecht, 2012; Marais *et al*., 2015; Mashela *et al*., 2017b; Onkendi and Moleleki, 2013). Approximately 453 plant-parasitic nematodes have been identified in potato-producing regions of South Africa (Marais *et al*., 2015), with 23% identified species being in the Eastern Cape Province (Marais and Swart, 2007). In one such survey, an estimated one million root-knot nematodes per 50 g potato roots were recorded (Mtshali *et al*., 2002). However, survey data in Limpopo Province are scanty (Marais *et al*., 2015). The major survey up to date showed that only three soil samples were collected from Limpopo Province for plant-parasitic nematode identification (Marais *et al*., 2015). As such, there exists a gap in knowledge concerning the diversity of plant-parasitic nematodes that are associated with potato in Limpopo Province. Therefore, the objective of this study was to determine the diversity and abundance of plant-parasitic nematodes associated with potato in Limpopo Province, South Africa. The null hypothesis was that the diversity and

abundance of plant-parasitic nematodes associated with potato in Limpopo Province, would not be different to those in other potato-producing regions of South Africa.

3.2 Materials and methods

3.2.1 Description of the study site

Sampling procedures

A total of 30 farms from four of the five district municipalities of Limpopo Province, South Africa namely, Capricorn, Mopani, Waterberg, and Greater Sekhukhune, were sampled to assess the diversity of nematodes associated with potato crop (Figure 1).

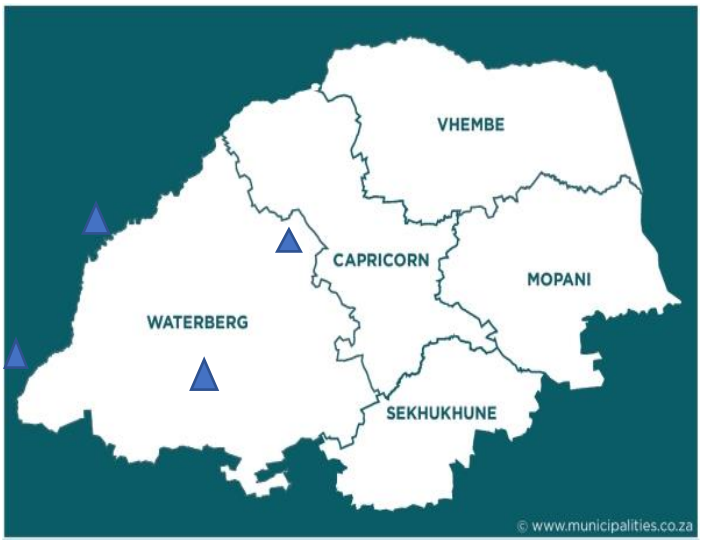


Figure 3.1 Limpopo Province map showing the ditricts (▲) from which soil samples were collected from potato fields for plant-parasitic nematode analysis (www.municipalities.co.za)

Overall, the farms were within the 23°30′89″ and 24°83′35″ latitudes, along with 28°29′94″ and 30°71′60″ longitudes. The farms were situated in the semi-arid region of the Province, with mean annual rainfall of less than 500 mm. All the farms were

under sprinkler irrigation systems. The sizes of the farms sampled ranged from one to five ha.

3.2.2 Data collection

Soil samples

Data were collected during autumn from 15 March to 11 April 2019. Most plant nematodes in various crops occur within the rhizosphere at the soil depth ranging from a few cm below the soil surface to 25-cm soil depth (Van Bezooijen, 2006). Approximately 10 core soil samples were randomly collected per hectare in a zigzag-sampling pattern using a 2.5-cm-diameter soil auger at a depth of 15-20 cm. The soil samples from each farm were individually placed in clear plastic/ polythene bags and transported to the laboratory in a cooler box. Prior to extraction of nematodes, the samples were kept in the cold room at 4˚C for a week. Thereafter, samples from each farm were thoroughly mixed using a 120 L concrete mixer (Turner Morris, Pretoria) to form a composite sample. A 250 ml subsample was taken from the composite sample for nematode extraction, identification, and enumeration.

Assaying nematodes

Nematodes were extracted using the sugar centrifugal flotation method (Jenkins, 1964; Marais *et al*., 2017). Briefly, the 250 ml subsample was placed in a 4-L bucket, which was half-filled with tapwater and the mixture stirred to ensure that soil particles were suspended in water. After the swirling had ceased, the aliquot was poured through a top-down nested 75- and 25-µm opening sieves. Collected nematodes in the 25-µm sieve were washed into 50 ml plastic beakers for temporary storage in the cold room at 4˚C for not more than six days. In the laboratory, the stored material was washed into 50 ml plastic centrifuging tubes. Approximately 5 g kaolin ($Al_2Si_2O_5(OH)_4$)

was added into each centrifuge tube containing nematodes suspended in water so that the nematodes could be forced to the bottom of centrifuge tubes during centrifuging (Coolen and D'Herde, 1972; Marais *et al*., 2017). The tubes were swirled for 30 s to evenly mix the contents. The suspension was then centrifuged at 1 800 RPM for four minutes using the Thermo Fisher Scientific Heraeus Multifuge × 3 Centrifuge (Terra Universal, United States of America). Thereafter, the supernatant was decanted and discarded leaving soil particle sediments containing nematodes at the bottom of the tube. Approximately 40 ml sugar solution, prepared by adding 624 g sugar into 1 L tapwater, was poured into each centrifuge tube. The sugar solution and sediment were thoroughly stirred with a spatula and centrifuged at 1 800 RPM for 3 min (Marais *et al.*, 2017). After the centrifugal flotation was complete, the supernatant containing the nematodes was decanted through a 25-µm aperture sieve and the contents rinsed thoroughly using tapwater to remove sugar. The nematodes were washed into a 50 ml plastic tube up to the 10 ml mark.

Morphological and anatomical traits were used for nematode identification (Heyns, 1971). The nematodes were identified to genus level and counted using a ZEISS Stemi 508 Greenough Stereo Microscope (Berlin, Germany) up to a magnification of × 100 (Marais *et al*., 2017). Before identification and counting, the contents were mixed thoroughly by shaking. A pipette was used to withdraw 2 ml aliquot for nematode identification and counting. The aliquot was placed on a clean and clear De Grisse counting dish (De Grisse, 1963). Two aliquots with equal volume were counted per sample and the average of the combined aliquot score was used to determine the nematode population densities per soil sample.

3.2.3 Data analysis

Nematode data obtained from the survey was used to compute mean population densities, frequency of occurrence, relative abundance and prominence values using the following formulas in Microsoft Excel 2013:

$$\text{Mean population densities} = \frac{\text{mean number of individuals of a particular species in 250 ml soil sample}}{\text{number of positive samples}}$$

$$\text{Frequency of occurrence} = \frac{\text{number of samples positive for a genus}}{\text{total number of samples}} \times 100$$

$$\text{Relative abundance} = \frac{\text{total number of individuals of a particular species in positive samples}}{\text{total number of samples}}$$

Prominence values = Mean population densities × (√Frequency of occurrence /10) (Almohithef *et al*., 2018; De Waele and Jordaan, 1988).

The nematode data were further subjected to Principal component analysis (PCA) using XLSTAT 2019 software to determine nematode diversity within the Province and to determine which species were prevalent. Principal component analysis was used because the nematode data obtained showed high variability and was not normally distributed.

3.3 Results

Approximately 83% of the potato farms sampled were associated with at least one plant-parasitic nematode species (Appendix 3.1). A total of ten genera, namely, *Scutellonema*, *Helicotylenchus*, *Telotylenchus*, *Rotylenchulus*, *Paratylenchus*, *Tylenchorhynchus, Criconema*, *Nanidorus*, *Meloidogyne* and *Pratylenchus* species were identified (Table 3.1).

Table 3.1 Mean densities (MD), frequency of occurrence (FO), relative abundance (RA) and prominence value (PV) of plant-parasitic nematodes per 250 ml soil sample collected from 30 smallholder potato farms in Limpopo Province.

Genera	MD[a]	FO[b] (%)	RA[c]	PV[d]
Scutellonema	56.62	43.33	24.10	36.86
Helicotylenchus	67.50	46.67	31.50	46.45
Paratylenchus	57.50	6.67	3.83	14.98
Tylenchorhynchus	15.00	3.33	0.50	2.74
Meloidogyne	201.57	46.67	94.07	138.00
Telotylenchus	59.50	26.67	15.87	30.73
Rotylenchulus	56.22	23.33	13.13	27.05
Criconema	56.50	26.67	15.07	29.44
Nanidorus	22.50	6.67	1.50	5.94
Pratylenchus	35.67	10.00	3.57	11.38

[a] Mean population density is the mean number of individuals of a particular species in 250 ml soil sample/number of positive samples.

[b] Frequency of occurrence is the number of samples containing a nematode genus/number of samples collected multiplied by 100.

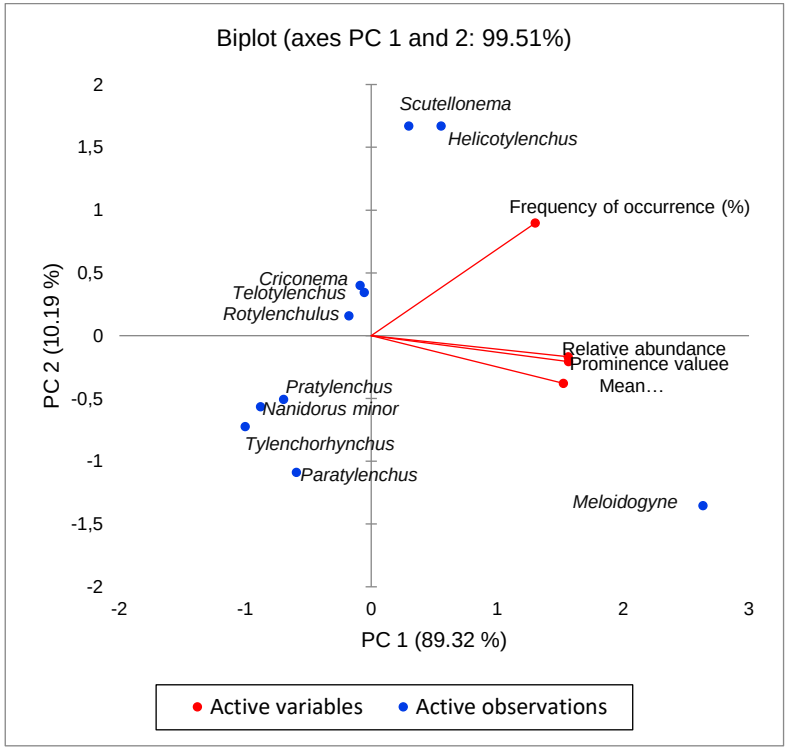
[c] Relative abundance the total number of individuals of a species in soil samples/total number of all samples.

[d] Prominence value is the mean densities multiplied by square root of frequency of occurrence divided by 10.

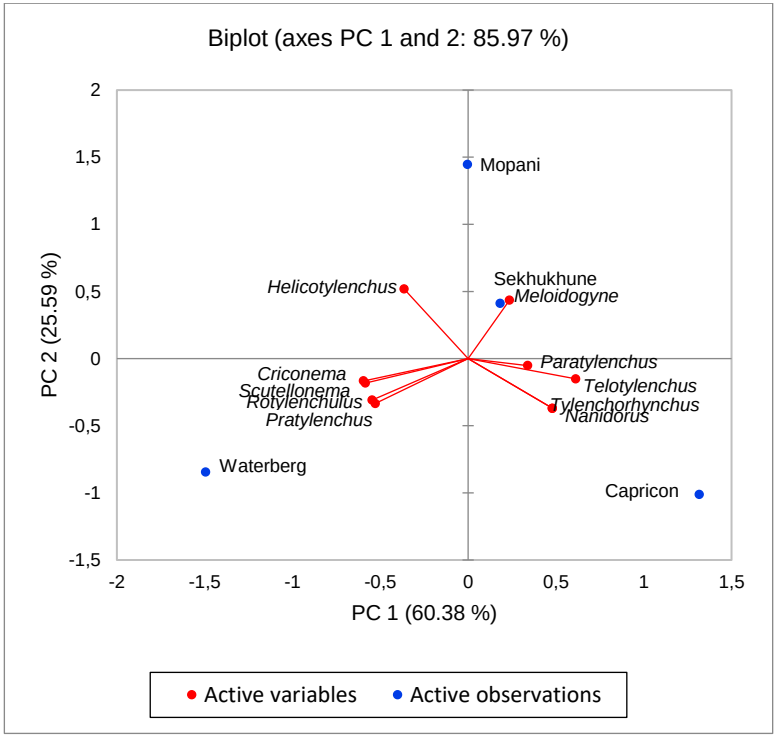
Meloidogyne species had the highest mean population density (Table 3.1). Additionally, *Meloidogyne* species was the most prevalent, followed by *Helicotylenchus and Scutellonema* species as exhibited by the frequency of occurrence values (Table 3.1). A similar trend was observed for relative abundance and prominence values. Conversely, *Tylenchorhynchus* and *Nanidorus* species were the least prevalent nematodes, with the lowest mean population densities, frequency of occurrence, relative abundance and prominence value (Table 3.1).

Total variation of the data sets was explained using two PCA components, PC1 (89.32%) and PC2 (10.19%), which had a cumulative variability of 99.51% (Figure 3.2a). The PC1 was positively correlated to mean densities, frequency of occurrence, relative abundance, and prominence value. Based on the Person correlation matrix, the active variables were strongly correlated to each other ($r \geq 0.66$) and were therefore clustered together.

The nematode genera were grouped into four assemblages. *Meloidogyne* species were positively correlated to PC1. Spiral nematodes (*Helicotylenchus* and *Scutellonema* species), *Telotylenchus* and *Criconema* species were strongly correlated to PC2. The spiral nematodes were grouped separately from *Telotylenchus* and *Criconema* species probably because other active variables influenced the grouping and were weakly correlated to all the calculated variables except for frequency of occurrence. The rest of the nematode genera were divided into two groups which were negatively correlated to PC1 (Figure 3.2a).



A



B

Figure 3.2 Biplots showing the mean densities, frequency of occurrence, relative abundance, and prominence value of the plant-parasitic nematodes associated with potato (A) and the distribution of nematodes in different districts in Limpopo Province (B).

The diversity of plant-parasitic nematodes sampled from different districts of Limpopo Province was shown on Figure 3.2b. The total variation of the nematode data set was explained by two principal components with a cumulative variability of 85.97%. Principal component 1 (60.38%) was positively correlated to *Tylenchorhynchus*, *Telotylenchus* and *Nanidorus* species, but it was negatively correlated to *Rotylenchulus*, *Criconema*, *Scutellonema* and *Pratylenchus* species. The PC2 (25.59%) was positively correlated to *Helicotylenchus* and *Meloidogyne* species (Figure 3.2b). Based on the squared cosine values (Appendix 3.2), the *Paratylenchus* species was positively correlated to PC3. As such, *Paratylenchus* species were not correctly represented on the biplot (Figure 3.2b). The Capricorn and Waterberg districts were positively and negatively correlated to PC1, respectively. Mopani district was strongly correlated to PC2. Sekhukhune district was positively correlated to PC3, based on the squared cosine value (Appendix 3.3).

3.4 Discussion

Based on the results, 83% of the potato farms sampled were associated with different genera of plant-parasitic nematodes with different population densities. Approximately 17% of the farms sampled were free of plant-parasitic nematodes, which could imply that the population densities of nematodes in those fields were too low to be detected by the sampling procedure used (Swart and Marais, 2017). Absence of plant-parasitic nematodes in soil samples could be attributed to many other factors such as the prevailing environmental conditions, edaphic factors, crop production practices, applied nematode management strategies and survival strategies which nematodes adopt under gradual adverse conditions (Nielsen *et al*., 2014).

The 10 identified nematode genera were previously reported in other potato-producing regions in South Africa (Engelbrecht, 2012; Marais *et al*., 2015), West Africa (Coyne *et al*., 2003) and globally (Lima *et al*., 2018). All the genera observed in this study were recorded for the first time in association with potato fields in Limpopo Province except for *Meloidogyne and Pratylenchus* species (Marais *et al*., 2015; Onkendi and Moleleki, 2013).

Root-knot nematodes and spiral nematodes (*Helicotylenchus* and *Scutellonema* species) were prevalent in most soil samples as exhibited by high frequency of occurrence. Conversely, *Meloidogyne* species frequency of occurrence was higher than that of spiral nematodes in potato-producing regions of the Northern Cape Province, South Africa (Marais and Swart, 2001). Information pertaining to the economic damage of *Helicotylenchus* species on potato or any other vegetable crops is scanty (Almohithef *et al*., 2018). However, *Helicotylenchus* species could be of great economic importance in potato production in the future (Lima *et al*., 2018) since they are K strategist nematodes that are least affected by environmental disturbances.

Notably, *Scutellonema* species were previously identified in various vegetable crop fields in Limpopo Province (Marais and Swart, 2002) and in potato fields in other regions of South Africa (Engelbrecht, 2012; Marais *et al*., 2015). However, there is little to no documentation on the pathogenicity of this nematode species on potato in South Africa. Nonetheless, *Scutellonema* species were identified as a potential threat to the potato-production industry in West Africa (Coyne *et al*., 2011; Mwamula *et al.*, 2015). Potato cultivated on soil-infested with the yam nematode (*Scutellonema bradys*

Steiner and LeHew), exhibited cracked, rotten, or small tubers, which were undesirable for fresh or processing markets (Coyne *et al*., 2011).

The prevalence of *Meloidogyne* species in agricultural soils and their effects on potato tuber yield and quality have been widely reported (Almohithef *et al*., 2018; Mokbel, 2014; Nakato *et al*., 2014; Onkendi and Moleleki, 2013). Similarly, *Meloidogyne* species were the most prevalent in potato fields that were sampled in North West Province, South Africa (Engelbrecht, 2012). A survey conducted in all Provinces in South Africa, except Eastern and Western Cape Provinces, showed that 81% of the sampled potato fields were infested with *Meloidogyne* species (Jones *et al*., 2017). However, the damage threshold of *Meloidogyne* species associated with potato in South Africa has not been established (Jones *et al*., 2017). In a bid to fully utilise the field and maximise on profit, smallholder potato farmers generally practice crop rotation or sequencing. Unfortunately, unbeknown to most farmers the crops that are included in these cropping systems are good host to root-knot nematodes (Mashela *et al*., 2017b). The inclusion of crops that are hosts to *Meloidogyne* species in cropping sequences probably proliferated the prevalence of root-knot nematodes since they are extremely polyphagous (Garcia and Sanchez-Puerta, 2012).

In this study, *Meloidogyne* and *Helicotylenchus* species co-existed in the soil samples collected. Similar observations have been reported in other potato-producing regions in South Africa (Engelbrecht, 2012; Marias *et al*., 2015), in olive tree fields (Hamza *et al*., 2018), banana plantation fields (Altman, 2018; Araya *et al*., 2006), rice (Gnamkoulamba *et al*., 2018), soybean (Mbatyoti, 2018), and many other vegetable

crops (Pedroche *et al*., 2013). Generally, *Meloidogyne* and *Helicotylenchus* species do not co-exist since they feed at the same sites (Crow, 2017), with the former as sedentary endoparasite and the latter as an ecto-parasitic nematode (Subbotin *et al*., 2015). In sugar cane studies, *Helicotylenchus* species population densities increased by 23% which resulted in a 54% reduction in *Meloidogyne* species population densities subsequently increasing crop yield (Daneel *et al*., 2018). However, it is important to note that the interaction of different species depends on several factors (Gomes *et al*., 2014). Gomes *et al*. (2014) reported that *Meloidogyne* species had a greater proliferating capacity in guava seedlings than *Helicotylenchus* species and concluded that the former was suppressing the latter. However, although *Meloidogyne* and *Helicotylenchus* species had a similar frequency of occurrence in the current survey, the mean densities, relative abundance and prominence value of *Meloidogyne* species were at least three times greater than those of *Helicotylenchus* species. Hence, *Meloidogyne* species were overall more prevalent than *Helicotylenchus* species. Perhaps, the agricultural practices, edaphic factors and prevailing climatic conditions generally promoted the prevalence of *Meloidogyne* species instead of *Helicotylenchus* species in sampled potato fields. Also, *Meloidogyne* species as r strategist nematodes, has high reproductive capacity than *Helicotylenchus* species which are K strategist nematodes.

Stunt nematode (*Tylenchorhynchus* species) and stubby root nematode (*Nanidorus* species) were the least prevalent nematode species in the survey. These nematodes commonly occur in potato fields in South Africa and around the world (Marais *et al*., 2015; Strand, 2006), and can be of great economic importance at high infection rates (Lima *et al*., 2018). Incidentally, the *Nanidorus* species are good vectors of tobacco

rattle virus (Riga *et al*., 2009), which reduce tuber yield by causing corky ringspot (Manzanilla-López *et al*., 2004). The limited prevalence of *Nanidorus* species in potato-producing regions of Limpopo Province confirmed observations in North West Province, South Africa, where the population densities of the same nematode species were less prevalent in sampled potato fields (Engelbrecht, 2012). Notably, other virus-transmitting ecto-parasitic nematodes such as *Longidorus*, *Paralongidorus*, *Paratrichodorus*, *Trichodorus* and *Xiphinema* in the order Dorylaimida, were not detected in soil samples from all potato-producing regions of Limpopo Province.

The other yield- and quality- threatening nematodes that were found in the soil samples were the root lesion nematodes. Fortunately, in this survey, these nematodes occurred below the estimated severe damage threshold value of 50 specimens per 250 ml soil sample (Jones *et al*., 2017). The occurrence of lesion nematodes was also reported in other potato-producing regions of South Africa (Marais *et al*., 2015; Parkinson, 2015). However, information on whether these nematodes were above the damage threshold value or not was not provided. The pathogenicity and aggressiveness of the other plant-parasitic nematodes observed in this survey such as reniform nematodes (*Rotylenchulus* species), *Telotylenchus* and *Criconema* species have not been documented despite their occurrence in potato fields in other Provinces (Engelbrecht, 2012; Marais *et al*., 2015).

Based on the principal component results (Figure 3.2a), *Meloidogyne* species showed a positive correlation with the calculated active variables (frequency of occurrence, mean densities, relative abundance, and prominence value). This observation

confirmed the predominance of *Meloidogyne* species in potato fields. However, a survey conducted in North Dakota and Central Minnesota, USA, showed that *Pratylenchus* species were the most prevalent nematodes occurring in most of the sampled potato fields (Upadhaya, 2018). Generally, *Pratylenchus* species are the most economically important nematode pests of potato in temperate regions (Castillo and Vovlas, 2007), whereas thermophilic *Meloidogyne* species are the most common nematode pests in tropical regions (Tariq-Khan *et al*., 2017).

Although *Helicotylenchus*, *Scutellonema*, *Telotylenchus* and *Criconema* species were strongly correlated to PC2, these nematodes were grouped separately because the frequency of occurrence of spiral nematodes was greater than those of *Telotylenchus* and *Criconema* species as summarised in Table 3.1. *Tylenchorhynchus*, *Nanidorus*, *Pratylenchus* and *Paratylenchus* species were negatively correlated to frequency of occurrence, mean densities, relative abundance, and prominence value. Therefore, these plant-parasitic nematode species were less prevalent in the sampled potato fields. *Pratylenchus* species are common in most potato producing areas in South Africa, but normally occur in low population densities that cause little to no damage (Jones *et al*., 2017). Generally, the information obtained from this PCA validated the information previously presented in Table 3.1 in this chapter. Conversely, PC1 was positively correlated to *Tylenchorhynchus*, *Telotylenchus* and *Nanidorus* species, but it was negatively correlated to *Rotylenchulus*, *Criconema*, *Scutellonema* and *Pratylenchus* species (Figure 3.2b).

The Capricorn and Waterberg districts were positively and negatively correlated to PC1, respectively. Therefore, potato fields in Capricorn district were predominated by *Tylenchorhynchus*, *Telotylenchus* and *Nanidorus* species, whereas *Rotylenchulus*, *Criconema*, *Scutellonema* and *Pratylenchus* species commonly occurred in Waterberg district. The PC2 was positively correlated to *Helicotylenchus* and *Meloidogyne* species. Alternatively, the Mopani district was strongly correlated to PC2. As such, potato fields in this district were mostly infested with *Helicotylenchus* and *Meloidogyne* species.

Based on the squared cosine values, *Paratylenchus* species were positively correlated to PC3. Hence, it could not be correctly represented in the biplot (Figure 3.2b). Conversely, the squared cosine value of the active observations showed that the Sekhukhune district was also positively correlated to PC3. As such, *Paratylenchus* species predominately infested potato fields in Sekhukhune district. According to the results, different nematode species were prevalent in different districts. Therefore, nematode management strategies required should address the prevalent nematode species occurring in each district as opposed to using one management strategy as a one-size fits all strategy as is commonly the case in nematode management.

3.5 Synthesis of study and conclusion

The nematode diversity in the current study was described as abundance and rate of occurrence, whereas the major survey to date only mentioned the presence or absence of different plant-parasitic nematodes associated with potato in different potato-producing regions of South Africa, without quantifying them. A total of eight new

nematode genera (*Helicotylenchus*, *Rotylenchulus*, *Paratylenchus*, *Tylenchorhynchus*, *Scutellonema*, *Criconema*, *Nanidorus*, and *Telotylenchus* species) were recorded for the first time in association with potato fields in Limpopo Province. Although most of these nematode genera have been previously reported in other potato-producing Provinces, that was not the case in Limpopo Province because the samples collected in previous surveys were too few to detect other plant-parasitic nematode species. Furthermore, a huge knowledge gap about the pathogenicity or economic damage that some of these plant-parasitic nematode genera might cause on commercial potato cultivars in South Africa still exist.

Based on the results, *Meloidogyne* species occurred in most soil samples and they were closely followed by *Helicotylenchus and Scutellonema* species. *Meloidogyne* species were the most prevalent plant-parasitic nematodes as exhibited by the high mean population densities, relative abundance and prevalence value. On the other hand, *Tylenchorhynchus* and *Nanidorus* species were the least prevalent plant-parasitic nematodes in Limpopo Province. The PCA results substantiated the latter, clearly showing different nematode genera that were prevalent in different districts. However, similar observations were not reported in the latest major survey conducted in other potato-producing regions due to certain limitations that were addressed in the current study. The Waterberg district had the highest plant-parasitic nematode diversity (*Rotylenchulus*, *Criconema*, *Scutellonema* and *Pratylenchus* species), followed by Capricorn (*Tylenchorhynchus*, *Telotylenchus* and *Nanidorus* species), Mopani (*Helicotylenchus* and *Meloidogyne* species) and Sekhukhune districts (*Paratylenchus* species). Variations in nematode diversity could be due to many factors such as the prevailing climate, soil type, crop history and cultural practices in

the sampled fields. Information from this survey might be used to develop and implement nematode management programmes designed to reduce predominant plant-parasitic nematodes such as *Meloidogyne* species. Additionally, farmers can be advised on the crops that can be used in rotation with their main potato crop to prevent proliferation of yield threatening nematode species. Overall, findings from the survey supported the hypothesis which indicated that the diversity of plant-parasitic nematodes associated with potato in Limpopo Province, would not be different to those in other potato-producing regions of South Africa. In the next chapter, the researcher investigated the efficacy of nematode resistant plants in managing root-knot nematodes and their effects on soil health in potato-based cropping systems.

CHAPTER 4

RESPONSES OF NEMATODES, PLANT GROWTH AND SOIL HEALTH TO FOUR CROPPING SEQUENCES

4.1 Introduction

Worldwide, all major potato-producing regions are severely infested with plant-parasitic nematodes (Marais *et al*., 2015), with 46% being the root-knot nematodes, *M. javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood (Onkendi and Moleleki, 2013). Correspondingly, *Meloidogyne* species were the most predominant plant-parasitic nematodes associated with potato (*Solanum tuberosum* L.) in Limpopo Province (Chapter 3). Moreover, the predicted increase in temperature due to climate change could render *Meloidogyne* species very difficult to manage due to the shortening of nematode ontogenies (Van der Waals *et al*., 2013). Recently, Rashidifard *et al*. (2019) demonstrated that another thermophilic *Meloidogyne* species, *M. enterolobii* Yang and Eisenback, with the ontogeny of 15 days, is becoming widely distributed in South Africa (Collett, 2020).

Most smallholder potato farmers underestimate the damage caused by nematodes and usually confuse nematode damage symptoms with those of abiotic stress factors such as drought and nutrient deficiency (Holgado and Magnusson, 2012; McSorley, 2011). Since potato is a tuber crop, damage by *Meloidogyne* species goes beyond yield reduction, with the quality of tubers from infested fields being highly compromised, covered with galls and/or cracks and therefore, unmarketable. In some cases, the nutrient content of the plant can also be compromised (Lima *et al*., 2018),

thereby affecting the nutrient content of the tubers. Potato tubers are an excellent source of carbohydrates, vitamins (C and B1), phosphorus, sodium, magnesium, calcium and potassium (Lubis *et al*., 2018). Generally, potassium as an osmoticum ion, is highly sensitive to nematode damage (Mashela *et al*., 2016).

Previously, synthetic chemical nematicides were widely used to manage nematode population densities, with claims that *Meloidogyne* species were not a pest in potato production. However, after the withdrawal of the fumigant chemicals in 2005 from the agrochemical markets because of their inherent harm to the environment, non-target organisms and humans (Mashela and Pofu, 2016), sustainable nematode management strategies were increasingly investigated (Mashela *et* al., 2017b). Unfortunately, all available potato cultivars have no resistant genotypes to *Meloidogyne* species (Lima *et al*., 2018; Pofu and Mashela, 2017a). Consequently, nematode resistant crops included in potato-based crop sequences were viewed as having the potential to serve as a sustainable strategy for managing population densities of *Meloidogyne* species to below economic damage threshold levels (Mashela and Pofu, 2016). However, the challenge for this strategy was the existence of two nematode resistance-mechanisms, one pre- and the other post-infectional nematode resistance (Huang, 1985; Shigueoka *et al*., 2019).

In pre-infectional nematode resistance, the second-stage juveniles (J2) are not allowed to penetrate the root systems, but most stages, including J1 in eggs, could eventually enter the survival phase (Mashela and Pofu, 2016; McSorley, 2003), serving as a reservoir for successor crops infection. Sweet stem sorghum (*Sorghum*

bicolor (L.) Moench) is an example of a crop that exhibits pre-infectional nematode-resistance mechanism against root-knot nematodes (Mashela and Pofu, 2016). However, its inclusion in nematode management cropping systems has not been well-documented despite its great adaptability to temperate, tropical, and subtropical climates (Mangena *et al*., 2018). Sorgoleone ($C_{22}H_{29}O_4$), the pre-formed, nematicidal root exudate is responsible for the pre-infectional nematode-resistance mechanism exhibited by most sorghum varieties (Czarnota *et al*., 2003).

In some cases, J2 penetrate the root systems in post-infectional nematode resistance, but they are prevented from either moving through the root system or establishing the feeding sites, thereby resulting in failure to develop to maturity and reproduce. *Cucumis africanus* (L.F.) is an example of a post-infectional root-knot nematode resistant crop that is indigenous to Limpopo Province, South Africa (Ramatsitsi, 2018). The *C. africanus* plant inhibits the establishment of the feeding site, thus trapping the J2 within the root system until they die of starvation (Ramatsitsi, 2018). Previous studies showed that *C. africanus* plants produce cucurbitacin B ($C_{32}H_{46}O_8$), a carbon-rich tetracyclic triterpenoid with nematicidal properties (Pelinganga, 2013). However, the inclusion of *C. africanus* plants in nematode management cropping systems has not been well-documented. In most cases, when crops are included in a cropping sequence to manage a specific nematode species, it is important to also investigate the effect of these crops on the physical, chemical and biological properties of soil, collectively referred to as soil health (Collange *et al*., 2011). Ideally, a good nematode management strategy should not be detrimental to soil health, but should rather promote this attribute.

The inclusion of different crops in a cropping sequence, even over a short period of time, could have either positive or negative effects on soil health (McDaniel and Grandy, 2016; Sharma *et al*., 2010; Zhong *et al*., 2016). Soil health, as alluded to earlier, is a biological property of soil which is influenced by biological, chemical and physical attributes (Collange *et al.,* 2011). The concept is defined as the continued capacity of soil to function as a vital living system to sustain biological productivity and promote plant, animal and human health (Larkin, 2015). A healthy soil is associated with an extensive biodiversity, rapid nutrient mineralisation and high resilience and resistance to deterioration (Collange *et al*., 2011; Sharma *et al*., 2010). Soil biodiversity is generally defined as the variety of living organisms that occur within the test soil (Collange *et al*., 2011). In contrast, nutrient mineralisation refers to the degradation of organic compounds into inorganic minerals by micro-organisms. In general, soil with high resilience and resistance to deterioration is sufficiently stable to withstand exposure to degrading environmental pressures (Collange *et al*., 2011).

The participation of nematodes in various processes related to soil health, at different soil food web levels, makes them excellent indicators for soil health analysis under different agricultural management practices (Yeates *et al*., 1993). The latter could be due to the fact that nematode communities readily respond to agricultural practices such as irrigation, fertilisation, weeding, crop diversity, residue retention, tillage practice and chemical use (Ferris *et al*., 2012; Grabau and Chen, 2016; Ingham, 2016). Several studies confirmed the effectiveness of using free-living nematode communities as a tool for assessing soil health (Blair *et al*., 1997, Ferris *et al*., 2001; Li *et al*., 2016; Neher*,* 2001, Wang and Hooks, 2011). In most cases, nematode faunal

analysis and community indices were used to evaluate soil health status under different cropping systems.

The commonly used community indices are enrichment index (EI), structural index (SI), channel index (CI), plant parasitic index (PPI) and maturity index (MI) (Wang and Hooks, 2011). The EI and SI explain the availability of nutrients and complexity of the food web within an environment, respectively. The CI is used to determine the decomposition pathway prevailing in the soil ecosystem which can be bacteria- or fungi-dominated (Ferris *et al*., 2001). The PPI gives the proportion of herbivorous nematodes occurring within a sample.

The MI is a measure of soil stability or disturbance whereby high MI values represent undisturbed, mature or pristine environments, predominated with persister (K-strategist) nematodes and a few coloniser (r-strategist) nematodes and *vice versa* at low MI (Bongers and Bongers, 1998). Basically, the r- strategists are small nematodes that are highly responsive to nutrient enrichment, are intolerant to environmental disturbances, have a short generation time and produce many eggs (Bongers and Bongers, 1998). Inversely, the K-strategists are large nematodes which are tolerant to changes in the environment, have long ontogenies, with females having relatively lower fecundities (Bongers and Bongers, 1998).

The assessment of soil microbial populations, carbon source utilisation profiles and enzyme activity in mineralisation of nutrients such as carbon, phosphorus and nitrogen, can provide information on how small changes in agricultural practices such

as crop sequencing can influence soil health (Habig *et al*., 2018). A better understanding of how different cropping sequences affect soil health can help farmers in making informed decisions before adopting new agricultural practices (Navarrete *et al*., 2016). Globally, information on how different potato-based nematode management cropping systems influence soil health is still scarce. Similarly, less is known about the impact of increasing plant diversity in potato-based cropping systems in South Africa.

The objective of this study was three-fold, namely, to determine whether: (1) monocultural potato sequences would have any effects on population densities of *Meloidogyne* species, plant growth and soil health, (2) sequencing potato with a post-infectional nematode resistant crop like *C. africanus* would have any effects on population densities of *Meloidogyne* species , plant growth and soil health and (3) sequencing potato with a pre-infectional nematode resistant crop like sweet stem sorghum would have any effects on population densities of *Meloidogyne* species, plant growth and soil health. The null hypothesis was also three-fold, namely, (1) monoculture sequences of potato would not have any effects on population densities of *Meloidogyne* species , plant growth and soil health, (2) sequencing potato with a post-infectional nematode resistant crop like *C. africanus* would not have any effects on population densities of *Meloidogyne* species, plant growth and soil health and (3) sequencing potato with a pre-infectional nematode resistant crop like sweet stem sorghum would not have any effects on population densities of *Meloidogyne* species , plant growth and soil health.

4.2 Materials and methods

4.2.1 Description of the study area

The experiment was conducted under field conditions with the history of mixed population densities of *M. incognita* and *M. javanica*, at two separate locations, during autumn (February-April) and spring (August-October) 2017, 2018 and 2019. Fields were fallowed in winter (May-July) and summer (November-January). The experiment was conducted at the University of Limpopo (UL), South Africa (23°53′10″S, 29°44′15″E). Validation was done in space at the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOPI), Roodeplaat, South Africa (25°61′44″S, 28°35′45″E). The soil at the UL location was predominantly Hutton sandy loam (65% sand, 16% clay, 19% silt), with EC_e 0.148 dS/m; pH (H_2O) 7.99; 82 mg K, 50.7 mg P, 1 290 mg Ca, 10.1 mg Na, 327 mg per kg soil and Pi = 943 J2/250 ml soil sample. At the ARC location the soil comprised sandy loam (8% clay, 60% sand, 32% silt), with EC_e 0.244 dS/m; pH (H_2O) 8.11; 68 mg K, 37.1 mg/kg, 1 240 mg Ca, 18.2 mg Na, 393 mg Mg per kg soil and Pi = 578 J2/250 ml soil sample. The preceding crops at the UL and the ARC fields were tomato (*Solanum lycopersicum* L.) and Swiss chard (*Beta vulgaris* subsp. *vulgaris*), respectively. Generally, continuous cultivation of vegetables was practiced at both fields. Fields were sampled for the initial nematode population densities (Pi) by randomly collecting 10 soil samples from each field. The samples were composited, and nematodes were extracted from 250 ml soil subsample using the sugar centrifugal flotation method as previously described (Chapter 3). Extracted J2 were standardised in 100 ml plastic containers for identification and counting from 5 ml aliquot using a Leica Zoom 2000 stereomicroscope at × 45 magnification.

4.2.2 Treatments and research design

In each rotational sequence, the treatments, namely, potato-Velum (control), *C. africanus*, potato cv. 'Mondial G3' and sweet stem sorghum cv. 'Ndendane' were arranged in a randomised complete block design, replicated six times. Blocking was performed for the heterogeneous distribution of soil nematode population densities.

4.2.3 Procedures and cultural practices

Each field was divided into six major blocks measuring 11 m × 2 m. Each major block was subdivided into 2 m × 2 m subplots, separated by a 1 m pathway. Each subplot contained four rows with six plants per row. The intra-row and inter-row spacing were standardised to 0.3 m and 0.5 m, respectively. In Sequence 1, the treatments were SSS cultivar, potato, *C. africanus* and potato-Velum. In Sequence 2, potato was cultivated on all plots as the successor main crop. In Sequence 3, the treatments were as in Sequence 1, whereas in Sequence 4 sole potato crop was cultivated as in Sequence 2 (Figure 4.1).

Figure 4.1 Example of random crop arrangement in Sequence 1 (A) and sole potato cultivation in Sequence 2 (B) at University of Limpopo.

***Planting potatoes**:* In preparation for setting the tubers, the soil was dug using spades, and levelled using rakes. Approximately four 2-m-long, 25-cm-deep furrows with an inter-row spacing of 0.5 m were dug per subplot using hand-held hoes. Basal fertiliser application was done at planting using 10 g NPK 2:3:2 (26) + 5% S, + 5% Ca + 0.5% Zn per plant, the fertiliser was lightly covered with soil to prevent direct contact with seed tubers. Potato tuber seeds with approximately the same size, previously kept in the dark for a week at room temperature (25˚C) to encourage sprouting, were selected for setting. Tubers were placed into 25-cm-deep furrows at an intra-row spacing of 0.3 m under a straight line and covered with topsoil. After setting, subplots were irrigated for 5 h using a drip irrigation system that discharged 1 L water per hour. Velum Prime nematicide was applied at setting on potato-velum plots at 500 ml/ha in Sequence 1 and Sequence 3. Top dressing was done using 5 g LAN/drip hole at four weeks after emergency. The plants were then carefully ridged using hand-held hoes.

***Planting sweet stem sorghum**:* The soil was prepared using hand-held hoes, 2-m-long and 2.5-cm-deep furrows were dug in preparation of planting. Sorghum seeds were primed for 24 h in chlorine-free tapwater, with excess water blotted out using laboratory paper towel prior to sowing. Four seeds were sown in 2.5-cm-deep holes adjacent to the drip holes and covered slightly with soil. A standard intra-row and inter-row spacing of 0.3 m and 0.5 m, respectively, was used. After sowing, subplots were irrigated for 5 h using a drip irrigation system that discharged 1 L water per hour. Seedling emergence was at 95% at 10 days after sowing. Two weeks after emergence, seedlings were thinned to one plant per drip-hole and fertilised using 5 g

NPK 2:3:4 (30) per plant, with top dressing at 5 weeks after crop emergence comprising 10 g NPK 2:3:2 (26) + 5% S, + 5% Ca + 0.5% Zn per plant.

Planting *Cucumis africanus*: The seeds from *C. africanus* fruit were used to produce seedlings under greenhouse conditions for use in Sequence 1 and Sequence 3. The seeds were sown in seedling trays, filled with Hygromix (Hygrotech, Pretoria, South Africa) growing media. The trays were placed in a germination room set at 45°C and each tray was irrigated using 1.5 L tapwater, three times a day, to prevent the growing media from drying. The trays were moved to the greenhouse three days after seedling emergence where ambient day and night temperatures averaged 27 and 22°C, respectively. In the greenhouse, seedlings were irrigated every other day using a watering can. The seedlings were hardened-off for one week outside the greenhouse through exposing them to the sun and intermittent withdrawal of irrigation water (Mashela, 2017) at four weeks after emergence. Seedlings were transplanted into 3-cm-diameter and 5-cm-deep holes. Six seedling holes were made per row and each subplot had four rows, with 0.3 m × 0.5 m spacing. Approximately 5 g and NPK 2:3:4 (30) and 2 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca fertilisers were applied per plant at transplanting, with the first intended to supply both macro- and micro-nutrient elements, whereas the latter was mainly intended to supply Ca. After transplanting, the subplots were irrigated for 5 h using a drip irrigation system that discharged 1 L water per hour. The drip irrigation system, with an output of 1 L/h, was used for 1 h to irrigate the crops every other day for four weeks. The plants were top dressed using 10 g NPK 2:3:2 (22) per plant at five weeks after transplanting. Thereafter, irrigation was increased to 2 h every other day until harvest. Weeds were manually removed

when necessary, using hand-held hoes. Insect pests and diseases were managed as recommended for potato in commercial farming systems in South Africa (DAFF, 2010), with other crops receiving similar pesticide applications. Whiteflies (*Trialeurodes vaporariorum* Westwood) were controlled using Whitefly Insecticide from Efekto applied at 5 ml/5 L water. Preventative sprays against early blight and late blight were done once throughout the growing period using Tenazole 250 EW at 75 ml/100 L water and Mycoguard 720 SC at 1 L/500 L water/ha, respectively. Copper-Flow-Plus bactericide and fungicide was applied once at 50 ml/10 L water to manage late blight that appeared to persist on potato.

4.2.4 Collection of nematode and plant data

Data were collected at 56 days after emergency of potato seedlings. Plant nematode variables (J2 + eggs in root and J2 in soil) were collected from all sequences, whereas plant variables (tuber yield, chlorophyll content, plant height, dry biomass and stem diameter) were collected in Sequence 2 and Sequence 4 only. Data were collected using standard procedures, described below.

Nematode variables: Soil samples were collected to the 25-cm soil depth per plant within each subplot using a 5-cm diameter soil auger and then composited, followed by mixing in a 120 L concrete mixer (Turner Morris, Pretoria, South Africa). Thereafter, a 250 ml subsample per crop was taken from the composite sample and used for extraction of final nematode population densities (Pf) using the sugar centrifugal flotation method as previously described (Chapter 3).

Eggs and J2 from roots were extracted using the blending and sieving method (Hussey and Barker, 1973), followed by the sugar centrifugal flotation method (Marais *et al*., 2017). Briefly, roots of separate crops in each subplot were removed and immersed into a bucket half-full with chlorine-free tapwater, with slight shaking to remove excess soil. Roots were blotted dry using a paper towel and cut into small pieces (± 0.5 cm). The small pieces were mixed using hands and a 10 g subsample was removed from the composite root sample for nematode extraction. The subsample was put in a jar, where 1% NaOCl solution was added until the roots were submerged. The jar contents were emptied into the blender, approximately 100 ml water was added, and the sample was blend for 60 s using a 1.75 L Russel Hobbs domestic blender. The aliquot was decanted through a top-down nested 75- and 25-µm aperture sieves. The root fragments in the 75-µm sieve were thoroughly washed under running tapwater before throwing them away. The nematode suspension collected from the 25-µm sieve were washed into 50 ml plastic centrifuging tubes and separated from debris and soil particles using the sugar centrifugal flotation method (Marais *et al*., 2017). Nematodes were identified and quantified using a Leica Zoom 2000 stereomicroscope at × 45 magnification. The reproductive potential (RP) of nematodes was calculated as J2 + eggs / g fresh roots.

Plant growth variables: Plant variables data were collected from sequences where sole potato cultivation was practiced on all plots (Sequence 2 and Sequence 4). Chlorophyll content, stem diameter and plant height were measured a day before harvesting using a SPAD-502 chlorophyll meter (Konica Minolta, Beijing, China), digital Vernier caliper and 2-m-long ruler, respectively. Chlorophyll content was collected from five healthy mature leaves per plant and averaged. Tuber yield was

measured at harvest using a CBK 8H Bench Check Weighing Scale balance (Adam Equipment, Oxford, UK). Shoots were dried for 3 days at 72˚C using an AD750 Agri-Dryer (Dryers for Africa, White River, South Africa) prior to determination of dry biomass. An HCB 2202 Highland Portable Precision Balances (Adam Equipment, Oxford, UK) was used to measure dry shoot mass.

Nutrient element analysis in leaf tissues of the successor potato crop: Prior to harvest, five healthy mature leaves were harvested, prepared and dried as described by Paul *et al*. (2017) for shoots and ground through a 1-mm sieve on a Thomas Wiley Mill (Thomas Scientific, Swedesboro, USA). Nutrient elements in potato leaves were determined using the digestion method (Hoobin and Vanclay, 2012). Briefly, approximately 0.4 g ground leaf materials were digested in 75 ml vessel with 5 ml nitric acid (HNO_3) and 3 ml hydrogen peroxide (H_2O_2). The vessels were inserted in a microwave digester (Perkin Eimer, Titan MPS, Waltham, USA), which was set at 50-260°C for 46 min. Afterwards, the vessels were cooled under room temperature for 20 min, with samples decanted into 50 ml tubes. Deionised water was added in each of the tubes up to the 50 ml mark. The tubes were stored for 4 days in the cold room set at 4˚C to avoid evaporation prior to analysis. Potassium, P, Ca, Mg and Na in leaf tissues were determined from each sample tube using the Inductively Coupled Plasma Optical Emission Spectrometry (Shimadzu, ICPE-9000, Kyoto, Japan). The macronutrient elements were expressed as percentages, whereas Na was expressed as ppm.

4.2.5 Collection of soil health data

Soil health data were determined from one 250 ml soil subsample for various components as detailed below:

Soil organic carbon content: Soil organic carbon was quantified using the Walkley-Black method with a few modifications (Walkley and Black, 1934). Approximately 250 g soil was air-dried for 3 days and ground using a pistil and a motor. The ground soil was passed through a top-down 2- and 1 mm nested sieve to homogenise the soil. A 1 g subsample was taken from the sieved sample and put into a 500 ml conical flask. Approximately 10 ml potassium dichromate ($K_2Cr_2O_7$) solution 1 N and 20 ml concentrated sulphuric acid (H_2SO_4) were added into the flask. The contents were swirled for 2 min and the flask was left on the desk for 45 min for the reaction to complete. The suspension in the flask was diluted by adding 200 ml distilled water. Then 10 ml orthophosphoric acid (H_3PO_4) and 1 ml diphenylamine ($C_{12}H_{11}N$) indicator were added into the flask until a violet colour appeared. The contents in the flask were titrated against 0.5 N ammonium iron (II) sulphate $(NH_4)_2Fe(SO_4)_2$ until the colour changed from violet to bright green. The volume of $(NH_4)_2Fe(SO_4)_2$ at the endpoint was noted. A blank titration, where no soil was added was done using the same procedure as in soil sample analysis. The amount of $(NH_4)_2Fe(SO_4)_2$ used for the blank titration was also noted. The following equation was used to calculate the amount of organic carbon in the soil sample:

$$\text{Organic carbon \% in soil} = \left[\frac{(V_B\text{-}V_S)\text{ml} \times 0.003 \times 100}{2 \times M}\right] \times 1.3$$

where, V_B = blank titration reading; V_S = volume at endpoint of the sample reading and M = mass of soil sample used

***Bacterial functional diversity*:** The population and diversity of bacteria in the soil was determined using the Community Level Physiological Profiling (CLPP) method (Hill *et al*., 2000). Soil samples collected from the rhizosphere of each plant in a plot were composited and sieved using a 2 mm sieve to remove larger soil particles and plant debris prior to dilution. Approximately 10 g sieved soil subsample was placed in a 200 ml beaker, where 90 ml sterile distilled water was added, and the contents shaken. Portions of the samples were inoculated into Biolog EcoPlates™ (Biolog® Inc., Hayward, USA) containing 31 carbon sources and a control well (water only), in triplicate. A multichannel pipette was used to dispense 0.15 ml aliquots into each EcoPlate well. The plates were then incubated in the dark at 28°C. Respiration of carbon sources by microbial populations reduced the tetrazolium dye found at the bottom of each well. The rate of carbon source utilisation by the microbial populations caused colour changes from colourless to purple, which was measured using a Microplate reader (BioBase Biodustry, Shandong, China) set at 590 nm wavelength. Measurements were taken twice daily over a period of 5-10 days to determine the average well colour development (AWCD). The functional diversity of the soil microbial populations was determined using the amount and equitability of carbon substrates metabolised in the Biolog Ecoplates™ as indicators of diversity (Shannon-Weaver diversity index) and abundance (Evenness index), respectively (Garland and Mills, 1991). The Shannon-Weaver calculated as H' = - Σ[pi ln(pi)], where p*i* = a*i*/ Σa which is the proportional turbidity recorded in the *i* th well, a*i* = turbidity of the *i* th well and Σa = total turbidity of all sample wells (Lahav and Steinberger, 2001). The Evenness index (E) was calculated as E = H'/ ln S, where H' is the calculated Shannon-Weaver diversity index; ln is the natural logarithm of S; whereas S is the number of species.

***Soil enzyme activity*:** The ability of the soil microbial population to obtain carbon, phosphorus and nitrogen was evaluated by measuring activities of ß-glucosidase, acid phosphatase and urease in the soil. Approximately 250 g soil sample of each treatment crop was passed through a 2 mm sieve and dried at 40˚C for two days using an 80 L ProLab drying oven (ProLab Systems, Manama, Bahrain). Then the soil samples were stored in the cold-room at 4-6°C until analysis. ß-Glucosidase and acid phosphatase activities were analysed using the Eivazi and Tabatabai (1988) and Eivazi and Tabatabai (1977) protocols, respectively. To determine the activity of ß-Glucosidase and acid phosphatase, 1 g subsample from each composite sample was placed in 50 ml Erlenmeyer flask and 0.25 ml toluene, 4 ml modified universal buffer and 1 ml p-nitrophenyl- ß-D-glucoside solution (25 nm) were added. A stopper was placed on the flask and the contents thoroughly mixed. Then the sample was incubated for 1 h at 37°C in the dark using a Thermo Scientific™ Precision™ shaking water baths (Terra Universal, Fullerton, USA). Thereafter, 1 ml $CaCl_2$ solution (0.5 *M*) and 4 ml Tris buffer were added to the flask while swirling. Instead of tris buffer, 4 ml of NaOH solution (0.5 *M*) was added for the acid phosphatase protocol. The soil suspension was filtered through a Whatmann filter 2v. Glucosidase and acid phosphatase activities were calculated by determining the release of p-nitrophenyl using a DR3900 spectrophotometer (HACH, Randburg, South Africa) at a wavelength of 410 nm against a standard calibration curve produced by analysing different concentrations of p-nitrophenol. Glucosidase and acid phosphatase activities were determined separately.

The Kandeler and Gerber (1988) method was used to determine the activity of urease. Approximately 5 g soil subsample was placed in 100 ml Erlenmeyer flask and 2.5 ml

urea solution was added. Stoppers were placed on the flask and the samples were incubated in the dark at 37°C for 2 h using a shaking water bath. After incubation, 50 ml KCl solution was added to the flask while shaking for 30 min. The soil suspension was filtered through a Whatmann filter 2v. Approximately 1 ml filtrate was pipetted into 50 ml Erlenmeyer flask. Then, 9 ml distilled water, 5 ml NaOH solution and 2 ml sodium dichloroisocyanide ($C_3Cl_2N_3NaO_3$) solution were added to the flask. The mixture was cooled under room temperature for 30 min before determining urease activity by measuring the release of ammonia from the solution using a spectrophotometer set at 690 nm wavelength. Results of urea were calculated with reference to a standard calibration curve obtained from analysing different concentrations of urea (Habig *et al*., 2018).

***Nematode functional diversity*:** The soil samples were kept in the cold room at 4˚C for 10 days. Nematodes were extracted from 250 ml soil subsample using the sugar centrifugal flotation method as previously described (Chapter 3). The nematodes were washed into a 50 ml plastic tube up to the 15 ml mark. Thereafter, nematodes were killed and fixed using the hot fixation method in formalin-acetic acid prior to identification (Chapter 3).

4.2.6 Data analysis

The Shapiro - Wilk test was performed to determine the normality of distribution of the collected data (Ghasemi and Zahediasl, 2012; Shapiro and Wilk, 1965). The RP and log-transformed J2 in the soil were not normally distributed. Thus, the data were subjected to non-parametric Friedman test using the XLSTAT 2019 software, with data from each location analysed separately. The treatment means were compared at the

probability level of 5% using the Nemenyi test (Nemenyi, 1963; Pohlert, 2014). However, since data on plant growth variables and nutrient elements were normally distributed, the data were subjected to analysis of variance (ANOVA) using SAS 2010 software. Treatment means were compared at the probability level of 5% using Fischer's Least Significance Difference test. Potato plant growth data were obtained in Sequence 2 and 4, where sole potato cultivation was practiced after crops intended for use in crop rotation systems. Soil organic carbon, microbial diversity and enzyme activity data did not meet the assumptions of ANOVA in all sequences and therefore, data were non-parametrically evaluated through the principal component analysis (PCA) using XLSTAT 2019 software. The biplots obtained from PCA were used to interpret the data, with data from each location analysed separately.

Nematode genus data were analysed using the Ninja programme (Sieriebriennikov *et al*., 2014). Each nematode taxa were assigned to different trophic groups and appropriate coloniser-persister value (c-p value) were assigned to each nematode genera (Bongers and Bongers, 1998; Yeates *et al*., 1993). The c-p values were used to calculate MI, PPI, CI, EI, and SI. These indices were used to characterise the different nematode communities observed per treatment soil sample, in all sequences. The following formulas were used to calculate each index:

1. Maturity index (MI) = $\Sigma((v_i \times f_i)/n)$ where v_i and f_i are the c-p value and the frequency value of the toxon *i* in a sample, respectively, and n is the total number of individuals present in a sample (Bongers, 1999).

2. Plant-parasitic index (PPI) = $\Sigma(v_i \times f_i)/n$), where v_i is the cp-value of a family, f_i is the frequency of the family in a sample and n is the total number of individuals present in a sample (Bongers, 1999).
3. Shannon-Weaver diversity index (H') = $- \Sigma[P_i\ ln(P_i)]$, where P_i is the proportion of the n_i in the nematode community n (Briar *et al*., 2012; Pielou, 1977).
4. Channel index (CI) = 100(keFu2/(keBa1 + keFu2, where keFu2 is the enrichment weightings of fungivores with a cp-2 value (Fu2), having a coefficient of 0.8, whereas keBa1 is the enrichment weightings of the bacterivores with cp-1 value (Ba1), having a coefficient of 3.2 (Ferris *et al*., 2001).
5. Enrichment index (EI) = 100(e/(e + b), where e= Σkene, where ke and ne represent the structure of indicator weights and abundance of nematodes in those guilds, respectively, and *b* is calculated as $\Sigma k_b n_b$, where k_b and n_b are the weighted constant of the guild and the number of nematodes in that guild, respectively (Ferris *et al*., 2001).
6. Structure index (SI) = 100(s/(s + b), where s = Σksns, where ks and ns represent the structure of indicator weights and abundance of nematodes in those guilds, and *b* is calculated as described above (Ferris *et al*., 2001).

The calculated EI and SI indices were used to compute the faunal profile of the samples collected per sequence using Microsoft Excel 2010.

4.3 Results

4.3.1 Nematode variables

Nematode variables in root samples were expressed as reproductive potential (RP) and those in soil samples were expressed as population densities in order to be aligned with the analytical procedure.

Reproductive potential (RP): At the UL location, the RP values of *Meloidogyne* species were significantly higher in potato monoculture and sorghum-potato cropping sequences, but lower in *C. africanus*-potato and potato-(Velum)-potato cropping sequences in Sequence 1 and 4 (Table 4.1). The RP values of *Meloidogyne* species in potato monoculture differed significantly from that observed in *C. africanus*-potato and potato-(Velum)-potato, but were not significantly different from those observed in sorghum-potato cropping sequence (Table 4.1). Simultaneously, the RP values in sorghum-potato sequence were not significantly different from those observed in *C. africanus*-potato and potato-(Velum)-potato. There were no significant differences in RP values of *Meloidogyne* species in all cropping sequences in Sequence 2 (Table 4.1).

In Sequence 3, sorghum-potato and *C. africanus*-potato had the lowest RP values of the test nematode, whereas potato monoculture and potato-(Velum)-potato system had the highest RP values for the test nematode. The RP values of the test nematode in sorghum-potato were not different from those observed in *C. africanus*-potato, but differed from those observed in potato-(Velum)-potato and potato monoculture, which were not significantly different.

The RP values of the test nematode in potato-(Velum)-potato were also not significantly different from those in *C. africanus*-potato sequence (Table 4.1). Generally, significantly higher RP values of the test nematode were observed in autumn (Sequence 2 and 4), for each cropping sequence, across the sequences. Conversely, lower RP values of the test nematode occurred on crops in spring (Sequence 1 and 3) (Table 4.1).

Table 4.1 Reproductive potential of *Meloidogyne* species in potato monoculture (P–P), sweet stem sorghum-potato (SSS–P), *Cucumis africanus*-potato (C–P) and potato-(Velum)-potato (PV–P) cropping sequences at the University of Limpopo, during spring (Sequence 1 and 3) and autumn (Sequence 2 and 4).

Cropping sequence [x]	Sequence 1 [y]	Sequence 2	Sequence 3	Sequence 4
P–P	$592.33^{aC} \pm 52.04$	$6028.00^{A} \pm 192.16$	$3662.00^{aB} \pm 226.22$	$10463.33^{aA} \pm 1321.59$
SSS- P	$343.00^{abB} \pm 15.12$	$5692.33^{A} \pm 328.67$	$77.00^{cB} \pm 5.71$	$8975.67^{abA} \pm 1422.02$
C–P	$210.67^{bB} \pm 21.64$	$3931.67^{A} \pm 261.56$	$466.67^{bcB} \pm 28.23$	$5746.00^{bA} \pm 1008.31$
PV–P	$181.67^{bC} \pm 3.12$	$3739.33^{AB} \pm 220.50$	$1433.33^{abBC} \pm 117.36$	$5287.00^{bA} \pm 429.30$
P-value	0.05	NS	0.05	0.05

[x]Means ± standard errors sharing the same lowercase letter within cropping sequences (column) were not different ($P \leq 0.05$) within a sequence according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

[y]Means ± standard errors sharing the same uppercase letter across sequences (row) were not different ($P \leq 0.05$) according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

NS = Not significant at $P \leq 0.05$ according to the Friedman's Rank Test. Non-parametric test was used because data were not normally distributed according to Shapiro-Wilk test.

Cucumis africanus-potato and potato monoculture had significantly higher RP values, whereas potato-(Velum)-potato and sorghum-potato had lower RP values in Sequence 1 and 3 at the ARC (Table 4.2). There were no significant differences in RP values of the test nematode in *C. africanus*-potato and potato monoculture or in potato-(Velum)-potato and sorghum-potato. Also, the RP values of the test nematode in *C. africanus*-potato and potato-(Velum)-potato were not significantly different in Sequences 3, although it differed significantly with RP value on sorghum-potato (Table 4.2).

In Sequence 4, potato monoculture and sorghum-potato had the highest RP values, but *C. africanus*-potato and potato-(Velum)- potato had the lowest RP values. The RP values in potato monoculture were significantly different from those in *C. africanus*-potato and potato-(Velum)-potato. However, it was not significantly different from that in sorghum-potato crop sequence. The RP values of the test nematode in sorghum-potato, *C. africanus*-potato and potato-(Velum)-potato were not significantly different (Table 4.2).

Generally, significantly higher *Meloidogyne* species RP values were observed in autumn (Sequence 2 and 4), for each cropping sequence, across the sequences. Conversely, lower RP values occurred on crops in spring (Sequence 1 and 3) (Table 4.2).

Table 4.2 Reproductive potential of *Meloidogyne* species in potato monoculture (P–P), sweet stem sorghum-potato (SSS–P), *Cucumis africanus*-potato (C–P) and potato-(Velum)-potato (PV–P) cropping sequences at the Agricultural Research Council-Vegetable and Ornamental Plants, during spring (Sequence 1 and 3) and autumn (Sequence 2 and 4).

Cropping sequence [x]	Sequence 1 [y]	Sequence 2	Sequence 3	Sequence 4
P–P	$1411.67^{aBC} \pm 67.86$	$5646.67^{AB} \pm 166.53$	$767.33^{aC} \pm 31.20$	$9796.67^{aA} \pm 962.29$
SSS–P	$715.33^{abB} \pm 12.69$	$4768.00^{A} \pm 222.12$	$30.33^{cB} \pm 9.49$	$5147.33^{abA} \pm 1272.83$
C–P	$2624.33^{aA} \pm 165.96$	$4171.67^{A} \pm 182.20$	$291.33^{abB} \pm 27.25$	$3027.67^{bA} \pm 522.41$
PV–P	$333.67^{bB} \pm 44.44$	$3978.67^{A} \pm 153.37$	$149.33^{bcB} \pm 12.66$	$2221.67^{bA} \pm 365.85$
P-value	0.05	NS	0.05	0.05

[x]Means ± standard errors sharing the same lowercase letter within cropping sequences (column) were not different ($P \leq 0.05$) within a sequence according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

[y]Means ± standard errors sharing the same uppercase letter across sequences (row) were not different ($P \leq 0.05$) according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

NS = Not significant at $P \leq 0.05$ according to the Friedman's Rank Test. Non-parametric test was used because data were not normally distributed according to Shapiro-Wilk test.

Soil nematode population densities: The soil nematode population densities of *Meloidogyne* species in potato monoculture and sorghum-potato plots were significantly higher than in *C. africanus*-potato and potato-(Velum)-potato plots in all sequences at the UL (Table 4.3). However, the soil nematode population densities were not significantly different in potato-(Velum)-potato, *C. africanus*-potato and sorghum-potato plots in Sequence 2, 3 and 4. Additionally, the soil nematode population densities in sorghum-potato and potato monoculture plots were not significantly different in Sequence 2, 3 and 4 (Table 4.3). Generally, there was a decrease in *Meloidogyne* species soil nematode population densities across Sequence 1 to 3 in all cropping sequences. However, soil nematode population densities drastically increased in all cropping sequences in Sequence 4 (Table 4.3).

At the ARC location, soil nematode population densities of *Meloidogyne* species in potato monoculture and sorghum-potato plots were significantly higher than in potato-(Velum)-potato and *C. africanus*-potato plots in Sequence 1 and 4 (Table 4.4). However, soil nematode population densities of *C. africanus*-potato were not significantly different from the highest or the lowest soil nematode population densities. In Sequence 2, the soil nematode population densities in all plots were not significantly different (Table 4.4). In Sequence 3, sorghum-potato had the lowest soil nematode population densities which were not significantly different from that in C. africanus-potato and potato-(Velum)-potato plots but differed significantly from soil nematode population densities in potato monoculture plots (Table 4.4). The highest soil nematode population densities were recorded in Sequence 1, 2 and 4 of each cropping sequence, across sequences. However, low soil nematode population densities were recorded in Sequence 3 (Table 4.4).

Table 4.3 Nematode population densities per 250 ml of soil collected from potato monoculture (P–P), sweet stem sorghum-potato (SSS–P), *Cucumis africanus*-potato (C–P) and potato-(Velum)-potato (PV–P) cropping sequences at the University of Limpopo, during spring (Sequence 1 and 3) and autumn (Sequence 2 and 4).

Cropping sequence [x]	Sequence 1 [y]	Sequence 2	Sequence 3	Sequence 4
P–P	$310.00^{aB} \pm 61.05$	$290.00^{aBC} \pm 25.56$	$196.67^{aC} \pm 24.99$	$2646.67^{aA} \pm 186.95$
SSS- P	$370.00^{aA} \pm 112.40$	$183.33^{abAB} \pm 20.92$	$80.00^{abB} \pm 10.00$	$1390.00^{abA} \pm 94.06$
C–P	$175.00^{abAB} \pm 32.22$	$176.67^{abAB} \pm 21.55$	$20.00^{bB} \pm 4.47$	$550.00^{bA} \pm 61.05$
PV–P	$120.00^{bAB} \pm 44.12$	$106.67^{bB} \pm 21.71$	$60.00^{abB} \pm 10.33$	$643.33^{bA} \pm 61.84$
P-value	0.05	0.05	0.05	0.05

[x]Means ± standard errors sharing the same lowercase letter within cropping sequences (column) were not different ($P \leq 0.05$) within a sequence according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

[y]Means ± standard errors sharing the same uppercase letter across sequences (row) were not different ($P \leq 0.05$) according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

Non-parametric test was used because data were not normally distributed according to Shapiro-Wilk test.

Table 4.4 Nematode population densities per 250 ml of soil collected from potato monoculture (P–P), sweet stem sorghum-potato (SSS–P), *Cucumis africanus*-potato (C–P) and potato-(Velum)-potato (PV–P) cropping sequences at the Agricultural Research Council-Vegetable and Ornamental Plants, during spring (Sequence 1 and 3) and autumn (Sequence 2 and 4).

Cropping sequence [x]	Sequence 1 [y]	Sequence 2	Sequence 3	Sequence 4
P–P	$826.67^{aA} \pm 277.16$	$300.00^{AB} \pm 22.51$	$66.67^{aB} \pm 3.33$	$910.00^{aA} \pm 54.10$
SSS–P	$696.67^{aA} \pm 246.82$	$226.67^{AB} \pm 21.13$	$20.00^{bB} \pm 2.58$	$713.33^{aA} \pm 69.02$
C–P	$383.33^{abAB} \pm 30.29$	$240.00^{AB} \pm 11.55$	$30.00^{abB} \pm 4.47$	$450.00^{abA} \pm 21.13$
PV–P	$156.67^{bAB} \pm 32.42$	$183.33^{A} \pm 27.04$	$23.33^{bB} \pm 3.33$	$173.33^{bA} \pm 32.93$
P-value [z]	0.05	NS	0.05	0.05

[x]Means ± standard errors sharing the same lowercase letter within cropping sequences (column) were not different ($P \leq 0.05$) within a sequence according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

[y]Means ± standard error sharing the same uppercase letter across sequences (row) were not different ($P \leq 0.05$) according to the Friedman's Rank Test followed by the Nemenyi's post-hoc test.

NS = Not significant at $P \leq 0.05$ according to the Friedman's Rank Test. Non-parametric test was used because data were not normally distributed according to Shapiro-Wilk test.

4.3.2 Plant growth variables

Analysis of variance: At the UL location, highly significant ($P \leq 0.01$) treatment effects were observed on dry root mass and shoot mass, whereas significant ($P \leq 0.05$) treatment effects were observed on tuber yield, contributing 54, 6 and 18% in total treatment variation (TTV) on the respective variables. However, no significant treatment effects were observed on stem diameter, chlorophyll content and plant height (Appendix 4.1). At ARC, treatments had highly significant effects on dry root mass and tuber yield, contributing 89 and 72% in TTV on the respective variables. Nonetheless, no significant treatment effects were observed for dry biomass, stem diameter, chlorophyll content and plant height (Appendix 4.2).

At the UL location, highly significant treatment effects on Mg and Na in potato leaf tissues were observed, contributing 68 and 41% in TTV on the respective variables (Appendix 4.3). Nevertheless, treatments showed no significant effects on Ca, K and P content (Appendix 4.3). At ARC, highly significant treatment effects were observed on Ca content in potato leaf tissues, contributing 33% in TTV on the variable (Appendix 4.4). Significant treatment effects were observed on Mg and P content, contributing 35 and 50% in TTV on the respective variables. However, no significant treatment effects were observed on Na and K content in potato leaf tissues (Appendix 4.4).

Yield and morphological traits*:* At the UL location, potato-(Velum)-potato and *C. africanus*-potato had the highest marketable yield mass and dry biomass which were not significantly different from that in sorghum-potato. In contrast, potato monoculture had significantly low marketable yield mass and dry biomass which were also not

significantly different from that in sorghum-potato (Table 4.5). However, potato monoculture marketable yield mass and dry biomass were significantly different from that in potato-(Velum)-potato and *C. africanus*-potato (Table 4.5).

At the ARC location, potato-(Velum)-potato and *C. africanus*-potato had significantly high marketable yield mass, whereas potato monoculture and sorghum-potato had the lowest marketable yield mass (Table 4.6).

Nutrient elements: Potato-(Velum)-potato and *C. africanus*-potato cropping sequences had significantly high Mg content on potato leaf tissue compared to that in sorghum-potato and potato monoculture. Alternatively, potato monoculture had the highest Na content which was significantly different from that in *C. africanus*-potato, sorghum-potato, and potato-(Velum)-potato. However, there were no significant differences in *C. africanus*-potato, sorghum-potato, and potato-(Velum)-potato Na content (Table 4.7).

Potato leaf tissue from *C. africanus*-potato and potato-(Velum)-potato cropping sequences had significantly high Ca and P content while potato leaf tissue from potato monoculture had the lowest Ca and P content (Table 4.8). There were significant differences in Ca content of sorghum-potato and *C. africanus*-potato, but Ca content in potato-(Velum)-potato was not significantly different from that in sorghum-potato or *C. africanus*-potato. However, Ca content in potato monoculture was significantly different from that recorded in all the other cropping sequences.

Table 4.5 Marketable yield mass (MYM), dry biomass (DBM), chlorophyll content (CLC), stem diameter (SDM) and plant height (PHT) of the successor potato plants (cv. ′Mondial G3′) sown in Sequence 2 and 4 at the University of Limpopo.

Cropping sequence	MYM	DBM	CLC	SDM	PHT
P–P	$270.42^{b} \pm 19.62$	$26.56^{b} \pm 1.63$	38.93 ± 0.85	7.61 ± 0.23	48.59 ± 2.80
SSS–P	$293.09^{ab} \pm 17.63$	$27.54^{ab} \pm 1.67$	38.18 ± 0.86	7.27 ± 0.32	47.69 ± 3.26
C–P	$313.09^{a} \pm 10.59$	$27.88^{a} \pm 1.71$	37.58 ± 1.11	7.02 ± 0.28	45.50 ± 2.53
PV–P	$321.81^{a} \pm 16.93$	$28.79^{a} \pm 2.37$	38.21 ± 1.07	6.76 ± 0.27	46.94 ± 3.56
P-value	0.05	0.05	NS	NS	NS

NS = Not significant at P ≤ 0.05 according to Fischer's Least Significance Difference test. The means ± standard error sharing the same letter within a column are not significantly different (P ≤ 0.05) according to Fischer's Least Significance Difference test. Analysis of variance (ANOVA) was used since data met normality requirements and all the other assumptions for ANOVA.

Table 4.6 Marketable yield mass (MYM), dry biomass (DBM), chlorophyll content (CLC), stem diameter (SDM) and plant height (PHT) of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 at the Agricultural Research Council-Vegetable and Ornamental Plants.

Cropping sequence	MYM	DBM	CLC	SDM	PHT
P–P	190.26[b] ± 12.30	14.69 ± 1.11	37.35 ± 0.68	6.84 ± 0.27	36.07 ± 2.10
SSS–P	198.11[b] ± 11.67	14.14 ± 1.28	37.63 ± 0.81	6.51 ± 0.24	34.05 ± 1.73
C–P	232.63[a] ± 13.80	17.07 ± 1.49	37.23 ± 0.75	6.57 ± 0.35	36.59 ± 2.46
PV–P	238.22[a] ± 16.41	16.34 ± 1.32	37.98 ± 0.88	6.68 ± 0.20	33.19 ± 2.13
P-value	0.05	NS	NS	NS	NS

NS = Not significant at P ≤ 0.05 according to Fischer's Least Significance Difference test. The means ± standard error sharing the same letter within a column are not significantly different (P ≤ 0.05) according to Fischer's Least Significance Difference test. Analysis of variance (ANOVA) was used since data met normality requirements and all the other assumptions for ANOVA.

Table 4.7 Calcium, potassium, magnesium, phosphorus, and sodium in leaf tissues of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 at the University of Limpopo.

Cropping sequence	Ca (%)	K (%)	Mg (%)	P (%)	Na (ppm)
P–P	2.36 ± 0.05	3.91 ± 0.17	1.20^{b} ± 0.08	0.47 ± 0.02	4349.00^{a} ± 60.47
SSS–P	2.38 ± 0.10	3.76 ± 0.22	1.31^{b} ± 0.08	0.42 ± 0.02	4104.20^{b} ± 63.26
C–P	2.42 ± 0.17	3.35 ± 0.25	1.64^{a} ± 0.09	0.47 ± 0.02	4153.10^{b} ± 73.86
PV–P	2.69 ± 0.14	3.06 ± 0.31	1.68^{a} ± 0.11	0.43 ± 0.02	4114.60^{b} ± 24.42
P-value	NS	NS	0.05	NS	0.05

NS = Not significant at P ≤ 0.05 according to Fischer's Least Significance Difference test. The means ± standard error sharing the same letter within a column are not significantly different (P ≤ 0.05) according to Fischer's Least Significance Difference test. Analysis of variance (ANOVA) was used since data met normality requirements and all the other assumptions for ANOVA.

Table 4.8 Calcium, potassium, magnesium, phosphorus and sodium in leaf tissues of the successor potato plants (cv. ′Mondial G3′) sown in Sequence 2 and 4 at the Agricultural Research Council-Vegetable and Ornamental Plants.

Cropping sequence	Ca (%)	K (%)	Mg (%)	P (%)	Na (ppm)
P–P	$2.23^{c} \pm 0.09$	3.51 ± 0.17	$1.29^{b} \pm 0.04$	$0.41^{b} \pm 0.02$	4111.50 ± 53.42
SSS–P	$2.44^{b} \pm 0.06$	3.48 ± 0.14	$1.48^{ab} \pm 0.05$	$0.45^{ab} \pm 0.01$	4183.30 ± 33.44
C–P	$2.76^{a} \pm 0.13$	3.86 ± 0.16	$1.31^{b} \pm 0.05$	$0.46^{a} \pm 0.02$	4179.20 ± 60.93
PV–P	$2.62^{ab} \pm 0.09$	3.13 ± 0.12	$1.59^{a} \pm 0.07$	$0.45^{ab} \pm 0.01$	4211.50 ± 44.13
P-value	0.05	NS	0.05	0.05	NS

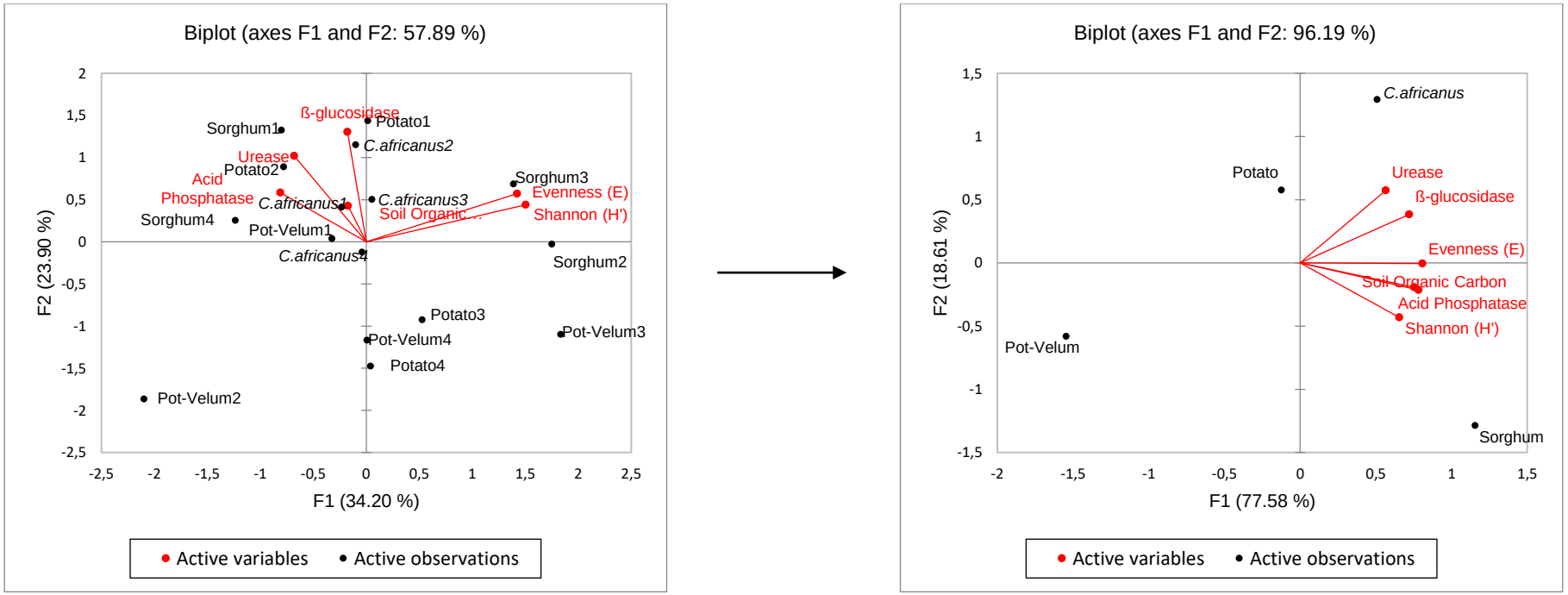
NS = Not significant at $P \leq 0.05$ according to Fischer's Least Significance Difference test. The means ± standard error sharing the same letter within a column are not significantly different ($P \leq 0.05$) according to Fischer's Least Significance Difference test. Analysis of variance (ANOVA) was used since data met normality requirements and all the other assumptions for ANOVA.

Phosphorus content in potato leaf tissue from potato monoculture, sorghum-potato and potato-(Velum)-potato cropping sequences were not significantly different (Table 4.8). Additionally, P content in potato leaf tissue from sorghum-potato, potato-(Velum)-potato and *C. africanus*-potato cropping sequences were not significantly different.

Potato-(Velum)-potato and sorghum-potato potato leaf tissue had significantly high Mg content, whereas potato leaf tissue from potato monoculture and *C. africanus*-potato cropping sequences had significantly low Mg content. Magnesium content in potato-(Velum)-potato was significantly different from that observed in potato monoculture and *C. africanus*-potato. However, it was not significantly different from sorghum-potato Mg content which was also not significantly different from potato monoculture and *C. africanus*-potato Mg content (Table 4.8).

4.3.3 Soil health

Soil organic carbon, microbial diversity, and enzyme activity: The effect of each treatment crop on soil health variables from Sequence 1 to 4 at UL was exhibited in Figure 4.2a. However, the effects of each treatment cropping sequence were best illustrated by combining data averaging over all four sequences (Figure 4.2b). The total variation of the data sets was explained by two components, PC1 (77.58%) and PC2 (18.61%), which had a cumulative variability of 96.19% (Figure 4.2b). Overall, PC1 was strongly influenced by all measured variables which were also strongly associated.



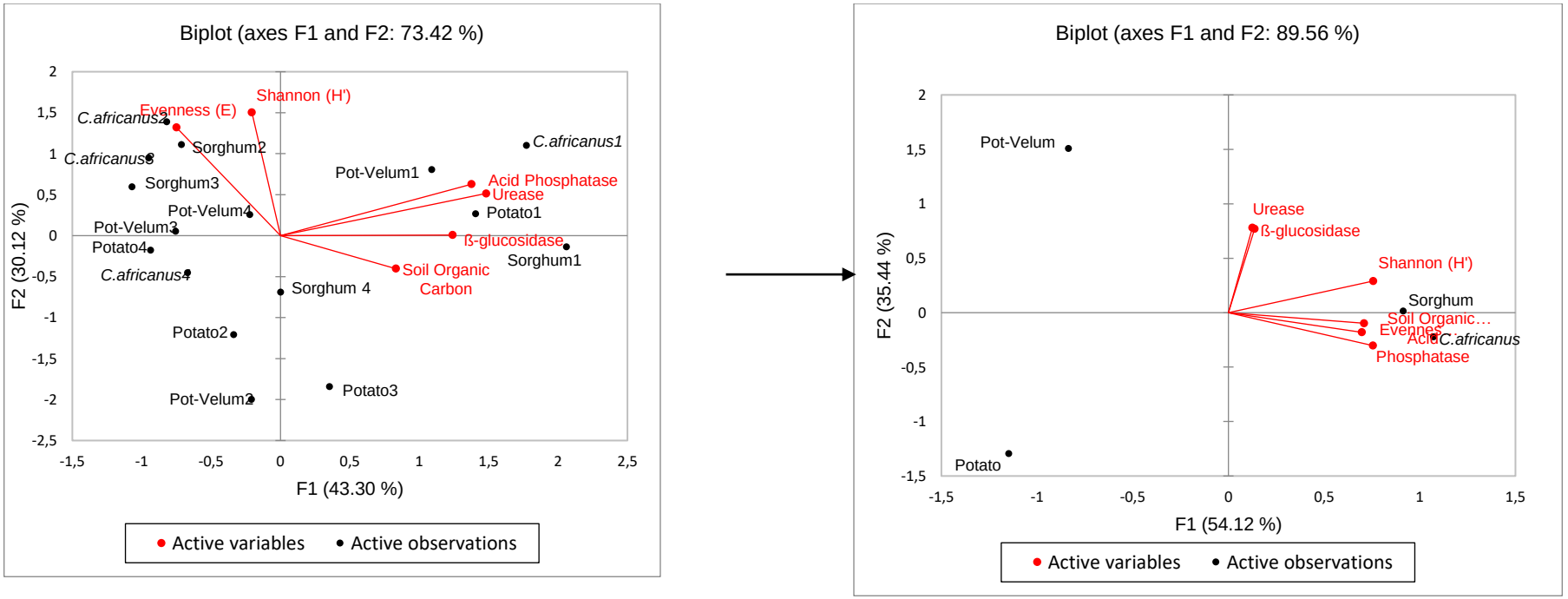
A B

Figure 4.2 Biplots showing how Potato = potato monoculture, Pot-Velum = potato-(Velum)-potato, *C. africanus* = *Cucumis africanus*-potato and sorghum = sorghum-potato cropping sequences affected soil organic carbon content, microbial diversity, and enzyme activity at the University of Limpopo.

The different cropping sequences were clearly divided using PC1. Based on the results, *C. africanus*-potato cropping sequence promoted urease and ß-glucosidase enzyme activity, whereas soil organic carbon, acid phosphatase enzyme activity and Shannon diversity were promoted by sorghum-potato cropping sequence (Figure 4.2b). Both *C. africanus*-potato and sorghum-potato cropping sequences positively correlated to evenness index. However, potato-(Velum)-potato and potato monoculture were negatively correlated to all measured variables, but there were placed in opposite quadrants (Figure 4.2b).

The effect of each treatment crop on soil health variables from Sequence 1 to 4 at ARC was exhibited in Figure 4.3a. Since the biplot had too much noise, the overall effect of each cropping sequence on soil health variables were considered instead (Figure 4.3b). Two components, PC1 (54.12%) and PC2 (35.44%), with a cumulative variability of 89.56% explained the total variation of the data sets (Figure 4.3b). The first principal component was strongly influenced by all the measured variables except for urease and ß-glucosidase enzyme activity which strongly influenced the second principal component.

The different cropping sequences were divided clearly by PC1 and PC2. *Cucumis africanus*-potato cropping sequence promoted soil organic carbon, acid phosphatase enzyme activity and evenness index, whereas Shannon diversity was strongly promoted by sorghum-potato cropping sequence (Figure 4.3b). In contrast, potato-(Velum)-potato and potato monoculture were negatively correlated with all the measured variables (Figure 4.3b).



A B

Figure 4.3 Biplots showing how Potato = potato monoculture, Pot-Velum = potato-(Velum)-potato, *C. africanus* = *Cucumis africanus*-potato and sorghum = sorghum-potato cropping sequences affected soil organic carbon content, microbial diversity, and enzyme activity at the Agricultural Research Council-Vegetable and Ornamental Plants.

Nematode communities as indicator for soil health: A total of 25 nematode genera with a c-p class ranging between 1 and 5 were identified from the soil samples obtained from S1 to S4 (Table 4.9). Generally, 48, 32, 12, 4 and 4% of the nematode species listed below (Table 4.9) were herbivorous, bacterivores, fungivores, predacious and omnivorous. The species *Tylenchorhynchus, Helicotylenchus, Scutellonema, Meloidogyne, Panagrolaimus and Aphelenchus* were the most abundant nematode species found across the sequences. In contrast, *Telotylenchus*, *Zeldia Elaphonema, Aphelenchoides, Eudorylaimus* and *Aporcelaimus* were the least represented nematode species.

Feeding type composition of nematode assemblage: Herbivores were the most dominating nematodes in soil samples collected from all sequences at UL and ARC. There were closely followed by bacterivores and fungivores (Figure 4.4). However, fungivores were common in Sequence 2 and 4 compared to Sequence 1 and 3. Omnivores were the least dominating nematodes which were found in Sequence 1 and 4 only. They commonly occurred in sorghum-potato cropping system, but were also found in potato monoculture and *C. africanus*-potato plots in Sequence 4 at ARC However, no omnivores were recorded in potato-(Velum)-potato plots at UL or ARC fields throughout the study (Figure 4.4).

Table 4.9 Functional diversity of nematodes found in four cropping sequences at University of Limpopo (UL) and at the Agricultural Research Council-Vegetable and Ornamental Plants (ARC).

Genera	X	Y	Z	SEQ 1	SEQ 2	SEQ 3	SEQ4
Criconema	0	3	HE	+		+	+
Criconemoides	0	3	HE	+	+		
Nanidorus	0	4	HE		+		
Tylenchorhynchus	0	3	HE	+	+	+	+
Xiphinema	0	5	HE			+	
Paratylenchus	0	2	HE			+	
Paratrichodorus	0	4	HE			+	
Helicotylenchus	0	3	HSE	+	+	+	+
Telotylenchus	0	2	HSE			+	
Rotylenchus	0	3	HSE	+	+		
Scutellonema	0	3	HSE	+	+	+	+
Meloidogyne	0	3	HS	+	+	+	+
Mesorhabditis	1	0	Ba	+	+	+	
Panagrolaimus	1	0	Ba	+	+	+	+
Acrobeles	2	0	Ba	+	+		
Acrobeloides	2	0	Ba	+	+		
Eucephalobus	2	0	Ba		+	+	+
Zeldia	2	0	Ba	+			

Table 4.9 Functional diversity of nematodes found in four cropping sequences at University of Limpopo (UL) and at the Agricultural Research Council-Vegetable and Ornamental Plants (ARC) (continued).

Genera	X	Y	Z	SEQ 1	SEQ 2	SEQ 3	SEQ4
Elaphonema	3	0	Ba		+		
Cephalobus	2	0	Ba		+	+	+
Aphelenchoides	2	0	Fu		+		
Aphelenchus	2	0	Fu	+	+	+	+
Ditylenchus	2	0	Fu	+		+	
Eudorylaimus	4	0	Pr	+			
Aporcelaimus	5	0	Om				+

[x] = coloniser-persister class, [y] = plant-parasitic class, [z] = Feeding type.

HE = herbivores-endoparasites, HSE= herbivores - semi-endoparasites, HS = herbivores - sedentary, Ba = bacterivores, Fu= fungivores, Pr = predators, Om = Omnivores and SEQ =sequence.

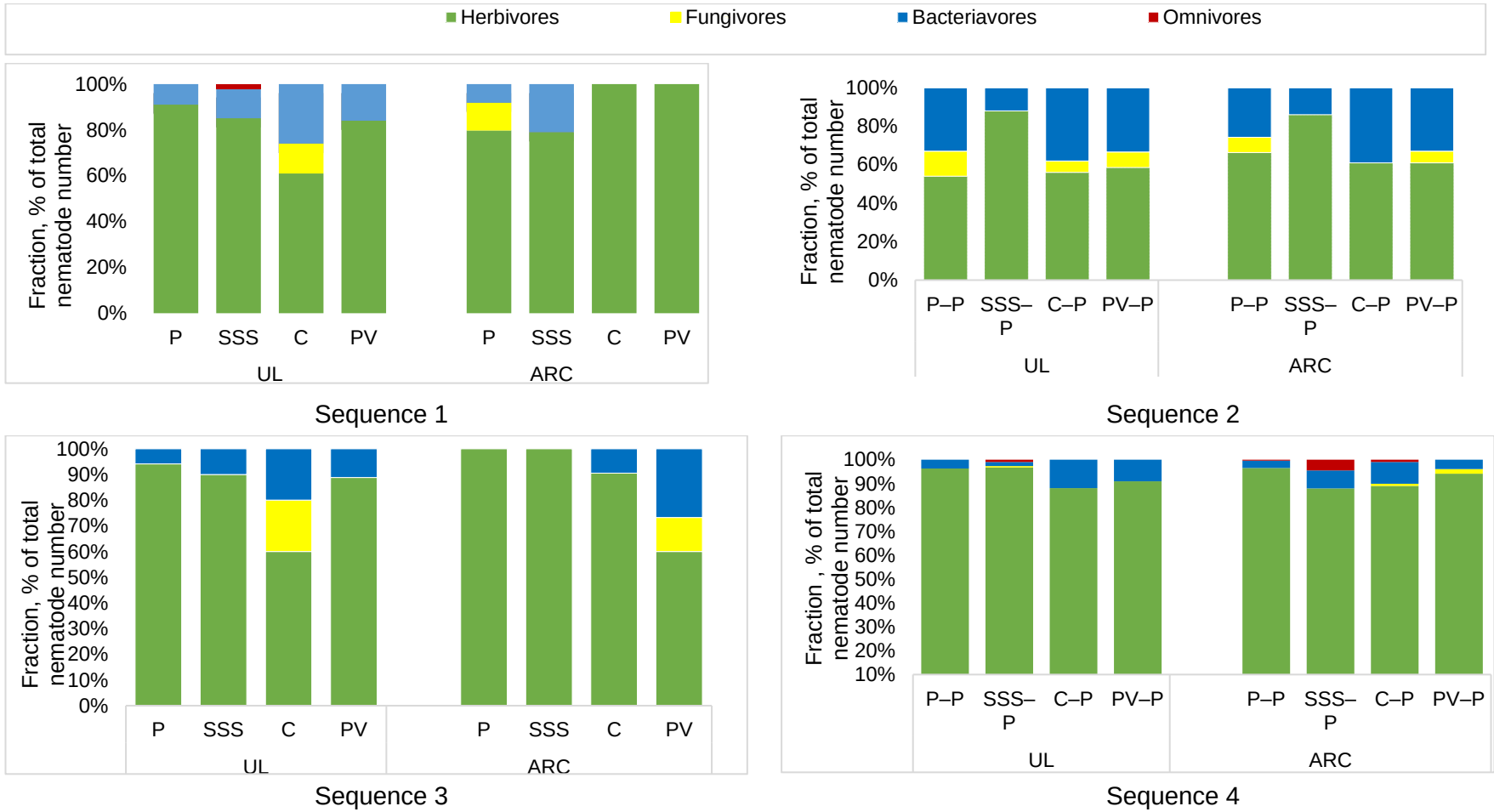
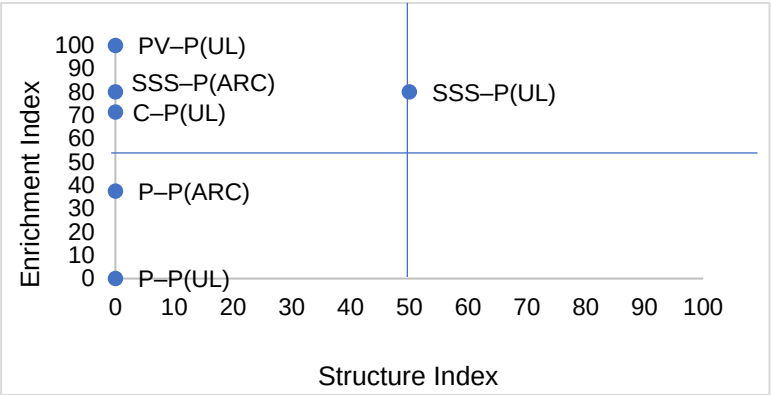


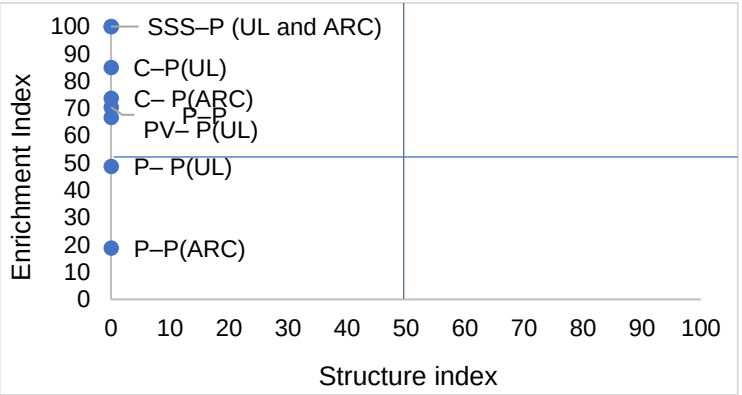
Figure 4.4 Feeding type composition of nematode assemblage of different treatment cropping sequences in each sequence at University of Limpopo (UL) and at the Agricultural Research Council (ARC), where P= potato, SSS = sweet stem sorghum, Ca = *Cucumis africanus,* PV = potato-(Velum)-potato. P–P = potato monoculture, PV–P = potato-(Velum)-potato, C–P = *Cucumis africanus*-potato and SSS–P = sweet stem sorghum-potato cropping sequences.

***Faunal profile*:** Plots sown with different cropping sequences throughout the study at UL and ARC were in either quadrant A or D. The plots sown with potato-Velum, sorghum-potato and *C. africanus*-potato from Sequence 1 to 3 were in Quadrant A, whereas potato monoculture plots were normally found in Quadrant DHowever, in Sequence 4 potato-(Velum)-potato, potato monoculture and *C. africanus*-potato were all grouped together in Quadrant D, whereas sorghum-potato was found in Quadrant C (Figure 4.5). Soil samples not shown in the diagrams had neither enrichment nor structure, hence they belonged to Quadrant D.

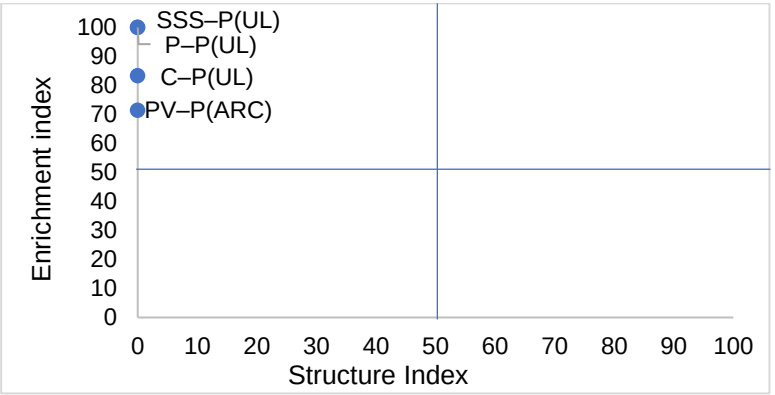
The MI of most cropping systems at UL and ARC were low (< 2) throughout the study (Table 4.10). However, sorghum-potato recorded moderate MI values in Sequence 4 at UL and ARC. Plant parasitic index was similar in all plots throughout the study except in Sequence 3 when *C. africanus* recorded the lowest PPI value at UL (Table 4.10). Generally, low CI values dominated most of the treatment plots at both UL and ARC. However, extremely high CI was recorded on potato monoculture (Sequence 1 and 2), *C. africanus*-potato and potato-Velum (Sequence 4) at ARC, whereas at UL, high CI value was observed on sorghum-potato in Sequence 4. The values for Shannon diversity index were very low (≤ 2). Therefore, low nematode species richness was observed on all the plots throughout the study.



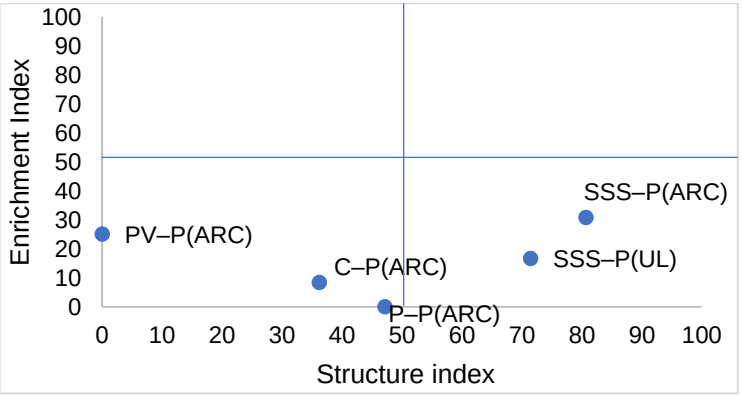
Sequence 1



Sequence 2



Sequence 3



Sequence 4

Figure 4.5 Nematode faunal profiles of soil samples collected from Sequence 1 to 4 at the University of Limpopo (UL) and Agricultural Research Council (ARC) fields. P–P = potato monoculture, PV–P = potato-(Velum)-potato, C–P = *Cucumis africanus*-potato and SSS–P = sweet stem sorghum-potato cropping sequences.

Table 4.10 Maturity index (MI), plant-parasitic index (PPI), channel index (CI) and Shannon diversity (H') of different cropping sequences at the University of Limpopo (UL) and at Agricultural Research Council-Vegetable and Ornamental Plants (ARC).

Sequence	Location	Treatment	MI	PPI	CI	H'
		P	2.00	3.00	0.00	0.93
		SSS	1.78	3.00	0.00	1.61
	UL	C	1.67	3.00	20.00	1.83
		PV	1.00	3.00	0.00	0.84
1		P	2.00	3.00	100.00	0.99
		SSS	1.50	3.00	0.00	1.41
	ARC	C	0.00	3.00	0.00	1.04
		PV	0.00	3.00	0.00	0.00
		P→P	1.86	3.12	33.30	2.00
		SSS→P	1.00	3.00	0.00	1.23
	UL	C→P	1.43	3.00	5.88	1.84
		PV→P	1.70	3.00	14.29	1.51
2		P→P	2.00	3.00	100.00	1.44
		SSS→P	1.00	3.16	0.00	1.45
	ARC	C→P	1.59	3.00	0.00	1.42
		PV→P	1.65	3.00	9.68	1.91
		P→P→P	1.00	3.00	0.00	1.00
		SSS–P-SSS	1.00	3.06	0.00	1.49

	UL	C→P→C	1.50	2.67	20.00	1.36
		PV→P→PV	2.00	3.50	0.00	1.68
3		P→P→P	0.00	3.00	0.00	0.00
		SSS–P-SSS	0.00	3.00	0.00	1.05
	ARC	C→P→C	2.00	3.00	0.00	1.61
		PV→P→PV	1.67	3.11	20.00	1.88
		P→P→P→P	2.00	3.00	0.00	0.49
		SSS→P→SSS→P	2.86	3.00	100.00	0.29
	UL	C→P→C→P	2.00	3.00	0.00	0.47
		PV→P→PV→P	2.00	3.00	0.00	0.30
4		P→P→P→P	2.38	3.00	0.00	0.30
		SSS→P→SSS→P	3.06	3.00	0.00	1.05
	ARC	C→P→C→P	2.25	3.00	100.00	0.66
		PV→P→PV→P	2.00	3.00	100.00	0.77

Where P = potato, PV = potato-(Velum), C = *Cucumis africanus* and SSS = sweet stem sorghum.

4.4 Discussion

4.4.1 Nematode variables

Reproductive potential (RP): In Sequence 1, potato monoculture and potato-(Velum) in the potato-(Velum)-potato sequence exhibited the highest and lowest *Meloidogyne* species RP, respectively at UL and ARC. The RP of the test nematode species was significantly higher in potato monoculture and sorghum in the sorghum-potato cropping

sequences than in *C. africanus* in the *C. africanus*-potato and potato-(Velum) in the potato-(Velum)-potato cropping sequences at UL. Although a Velum is a fungicide, it has been reported to reduce root-knot nematode damage in potato fields by 100% (Korayem *et al*., 2012). In this study, root-knot nematodes RP on post-infectional nematode resistant *C. africanus* plants (Ramatsitsi, 2018) were like that in potato-(Velum) treatment.

Post-infectional nematode-resistance mechanism is triggered when the second stage juveniles (J2) penetrate the root system of a plant (Huang, 1985). This mechanism is mainly associated with the hypersensitive response, which is clearly explained by the gene-for-gene model (Bent, 1996; Mashela *et al*., 2017a). Low RP value on *C. africanus* plants was reported by other researchers (Liu *et al*., 2015; Pofu *et al*., 2010; Pofu *et al*., 2012). As observed by Ramatsitsi (2018), the establishment of the feeding site was inhibited on *C. africanus* plants, but necrotic spots which trapped the J2 were observed. Generally, in active hypersensitive resistance, J2 are trapped and starved to death during movement from the penetration to the feeding sites before reaching maturity and reproducing (Huang, 1985; Ramatsitsi, 2018).

At the ARC location, *C. africanus* in the *C. africanus*-potato sequence and potato monoculture had significantly high RP values of the test nematode, whereas potato-(Velum) in the potato-(Velum)-potato and sorghum in the sorghum-potato sequences had lower RP values in Sequence 1. Although unexpected, root-knot nematodes were able to successfully infest and reproduce within the root system of *C. africanus* plants, which contradicted previous observations where the plant species was resistant to thermophilic

nematode species. The observation corroborates with the findings reported by Maleka (2021) who observed that the location was heavily infested with *M. enterolobii*. The failure to display post-infectional nematode resistant mechanism by *C. africanus* can also be attributed to delayed hypersensitive response as observed by Ramatsitsi (2018) on *C. africanus* and by others (Das *et al*., 2008; Silva *et al*., 2013), in other crops. The factors responsible for triggering delayed hypersensitive response are not fully understood (Mashela *et al*., 2017a), but have interactions with a wide range of abiotic and biotic stress factors (Silva *et al*., 2013; Williamson and Hussey, 1996). However, hypersensitive response is a delayed response by nature (Pofu, 2012). In cases where the response is delayed, the defence mechanism becomes weak and *vice versa* (Mashela *et al*., 2017a).

In other studies, nematode-resistance mechanism in plants was broken by biotic and abiotic stress factors such as whiteflies, aphids, and salinity (Maleka, 2021; Mashela and Nthangeni, 2002; Pofu *et al*., 2011). For example, whiteflies broke post-infectional nematode resistance in *C. africanus* (Pofu *et al*., 2011), whereas aphids broke pre-infectional nematode resistance in sweet stem sorghum (Maleka, 2021). Similarly, at temperatures above 32˚C root-knot nematode resistance in tomato cultivar with post-infectional nematode resistance was broken (de Carvalho *et al*., 2015; Noling, 2016). Under global warming, with predictions suggesting that average annual temperatures might increase by 1.7˚C in 2030 (Girvetz *et al*., 2019), most existing nematode resistant cultivars could face tremendous challenges from both abiotic and biotic factors. However, in the current study, *C. africanus* plants were not exposed to any known abiotic or biotic

stress factors, suggesting that the causes for delayed hypersensitive response were numerous.

Based on the current results, *Meloidogyne* species RP on sorghum in the sorghum-potato sequence was intermediate at UL and ARC in Sequence 1. Thus, the root exudates produced by the pre-infectional nematode resistant sorghum cultivar partially repelled or killed the target nematodes, with some of the nematodes managing to penetrate and reproduce within the root system. Similar observations were reported under field conditions when pre-infectional nematode resistant crops were cultivated, where RP values were high (Djian-Caporalino *et al*., 2019; McSorley and Gallaher, 1993; Pofu and Mashela, 2017b).

In Sequence 2, the RP of *Meloidogyne* species were not significantly different on all cropping sequences at both test locations, UL and ARC. Thus, root-knot nematodes managed to reproduce similarly on all cropping sequences when the successor potato crop was sown. Intrinsically, the successor crop had stimulatory effect on RP of the test nematode and subsequently nullified the treatment effect of the preceding crop. Similar findings were reported when different crops exhibiting different mechanisms of resistance were used to manage root-lesion (*Pratylenchus penetrans* Cobb) nematodes in potato fields (Belair *et al*., 2006) and reniform (*Rotylenchulus reniformis* Linford and Oliveira) nematodes in cotton (*Gossypium hirsutum* L.) fields (Stetina *et al*., 2007). Additionally, these results corroborate with those in other studies (Miller *et al*., 2006; Westphal, 2011), with the hypothesis that short crop sequences were sometimes not effective in managing

plant nematode population densities under field conditions where multiple nematode species occur naturally.

In Sequence 3 at the UL and ARC locations, potato monoculture and sorghum in the sorghum-potato sequence exhibited the highest and lowest RP values, respectively. The high RP value on nematode susceptible potato monoculture was expected. Similar observations were reported when nematode susceptible wheat (*Triticum aestivum* L.) (Williamson *et al*., 2013) and cotton (Davis *et al*., 2003) were monocultured. The low RP of the test nematode on sorghum in the sorghum-potato sequence could be attributed to sorgoleone ($C_{22}H_{29}O_4$), a root exudate released by most sorghum varieties with nematicidal properties (Czarnota *et al*., 2003; Sikora *et al*., 2005, Tibugari *et al*., 2019; Weston *et al*., 2013). Sorgoleone can either kill or repel root-knot nematodes prior to penetration in the root system of sorghum. Campos *et al*. (2006) demonstrated how sorgoleone reduced mobility and increased mortality of *M. javanica* J2, which subsequently reduced the RP values. In another study, sorghum sown on root-knot nematode infested soils showed no galls or J2 on the root system, thereby depicting the ability of sorghum cultivars to repel or kill nematodes within the rhizosphere (Mashela and Pofu, 2016).

The RP of the test nematode on sorghum in the sorghum-potato sequence was not significantly different from that of *C. africanus* in the *C. africanus*-potato sequence, but differed significantly from that in potato-(Velum) in the potato-(Velum)-potato sequence

at UL. As such, sorghum from the sorghum-potato sequence outperformed potato-(Velum) from the potato-(Velum)-potato sequence. Similarly, cotton-aldicarb exhibited high reniform nematodes RP values when compared to where resistant soybean (*Glycine max* L.) and maize (*Zea mays* L.) varieties were included in cotton-based cropping sequence (Davis *et al*., 2003). The inefficacy of synthetic chemical nematicides depends on a number of edaphic factors, chemical application method or rate, degradation of the chemicals by soil micro-organism, leaching beyond the root-zone and rate of volatilisation (Giannakou *et al*., 2005). At the ARC location, the RP of the test nematode on sorghum in the sorghum-potato sequence was not significantly different from that of potato-(Velum) in the potato-(Velum)-potato sequence, but differed significantly from that of *C. africanus* in the *C. africanus*-potato sequence. As such, sorghum in the sorghum-potato sequence slightly outperformed *C. africanus* in the *C. africanus*-potato sequence. Nevertheless, there were no significant differences in *Meloidogyne* species RP values on *C. africanus*-potato and potato-(Velum)-potato at both sites.

In Sequence 4, the RP of *Meloidogyne* species was significantly higher on potato monoculture and on potato in the sorghum-potato cropping sequence than on potato in the *C. africanus*-potato and potato-(Velum)-potato sequences at both locations. The low RP of *Meloidogyne* species on potato in the *C. africanus*-potato sequence, suggested that the preceding *C. africanus* treatment crop was effective in reducing the carry-over root-knot nematode population densities due to its ability to trap and kill J2, thus exposing the successor potato crop to lower infection levels. In contrast, high *Meloidogyne* species RP on potato in the sorghum-potato sequence suggested that sorghum plants were self-

protecting and less effective in reducing the carry-over nematode population densities. The observation corresponded with findings reported by others (Kratochvil *et al*.,2004; Mashela and Pofu, 2016) when sorghum-potato sequences were used to manage population densities of root-knot nematodes.

Generally, significantly higher RP values of the test nematode were observed in Sequence 2 and 4 during autumn, whereas lower RP values occurred on crops in Sequence 1 and 3 during spring. Generally, RP values were higher in autumn than in spring. This observation corroborates other findings whereby low nematode infection and reproduction were observed when sampling was done in spring compared to autumn (Ami *et al*., 2018; Kratochvil *et al*., 2004; Vela *et al*., 2014). In spring, the cooler soil temperature of the previous winter season could encourage nematodes to enter survival stages in eggs (McSorley, 2003). In most cases, crops cultivated in spring are exposed to low soil temperatures, thus the infection rate and proliferation of root-knot nematodes are low (Ami *et al*., 2018; Kratochvil *et al*., 2004; Vela *et al*., 2014). On the other hand, crops produced in autumn are exposed to high nematode infection rate since high soil temperatures and rainfall in summer tend to remain constant, thereby increasing juvenile hatch which in the presence of adequate food, juvenile develop to maturity and then reproduce (Ami *et al*., 2018; Kratochvil *et al*., 2004; Vela *et al*., 2014).

The general trend in the current study showed that soil population densities of *Meloidogyne* species in potato monoculture and sorghum-potato plots were significantly

higher than in *C. africanus*-potato and potato-(Velum)-potato sequences in all trials except in Sequence 2 at the UL location. Therefore, *C africanus* plants in the *C africanus*-potato sequence were more effective than sorghum cultivar in the sorghum-potato sequence and monoculture in reducing soil nematode population densities in Sequence 1,3 and 4. According to Huang (1985), J2 that penetrate the root system of post-infectional nematode resistant plants become trapped and die of starvation due to failure to feed. Due to the trapping, the soil nematode population densities are also reduced. Kimpinski *et al*. (2000) and Wang *et al*. (2007) demonstrated that the inclusion of post-infectional nematode resistant marigold (*Tagetes* species) cultivars in potato-based cropping system significantly reduced soil nematode population densities by 14%, probably due to the trapping of J2 in the roots. Similarly, post-infectional nematode resistant pepper (*Capsicum annuum* L.) crop reduced the carry-over of root-knot nematode population densities from 80 to 90% (Navarrete *et al*., 2016).

Generally, soil nematode population densities on sorghum in the sorghum-potato sequence were significantly higher than those on *C africanus* in the *C africanus*-potato and potato-(Velum)-potato sequences. The ineffectiveness of sorghum can be attributed to the fact that the toxic root exudates released into the rhizosphere to kill or repel nematodes can cause various stages of nematodes to enter survival stages (McSorley, 2003; Perry and Moens, 2011). In such cases, the pre-infectional nematode resistant crop is protected from nematode damage since the root exudates renders the plant-parasitic nematodes inactive. However, once the environmental conditions become favourable and a susceptible crop such as potato is cultivated, the dauers in eggs develop through

moulting to J2, which hatch and the nematode populations resurge prodigiously (McSorley, 2003).

Sorgoleone ($C_{22}H_{30}O_4$) released by the sorghum varieties as a root exudate can only persists in the soil for seven weeks or less before it is mineralised by soil micro-organisms (Dayan *et al*., 2010; Weston and Czarnota, 2001). Thus, the effects of crop sequencing can quickly diminish before the main crop is sown. The latter explains why nematode resurgence was observed on potato in the sorghum-potato sequences. Therefore, to improve the effectiveness of sorghum in controlling root-knot nematodes in cropping sequences the successor crop should be sown within the 7-week period after harvesting the non-host sorghum crop (Hookes *et al*., 2010).

In Sequence 2, *C africanus*-potato and sorghum-potato had similar effects on soil nematode population densities when potato was sown at UL. The observation showed that the successor crop nullified the treatment effects of the preceding crop and promoted similar root-knot nematode stimulatory effect. The nullification of treatment effects was also reported on potato and cotton fields infested by root-lesion (*Pratylenchus penetrans* Cobb) and reniform nematodes, respectively (Belair *et al*., 2006; Stetina *et al*., 2007). The observation suggested that a significant change in soil nematode population densities was not easily noticed upon initial introduction of the non- or poor-host crop in a cropping sequence (Brodie, 1996; Lima *et al*., 2018; Reddy, 2017). According to Davis *et al*. (2003), such observations normally occur where plants are cultivated in soils with high initial

nematode population densities of the target nematode or where a mixture of plant-parasitic nematodes affect the treatment crop e.g., under field conditions.

High and low soil nematode population densities were observed on potato in potato monoculture and potato-(Velum)-potato sequences, respectively, in all sequences at UL and ARC. However, in some studies, significantly high soil nematode population densities under potato monoculture were only reported after at least two years of monocropping (Crow *et al*., 2000; Eberlein *et al*., 2016; Oostenbrink, 1961). Such differences could be due to the differences in initial nematode population densities, the susceptibility of the cultivar to the test nematode, timing in sowing after harvesting the non-host or many other environmental factors that affect the host × nematode interaction. In contrast, potato in the potato-(Velum)-potato sequence recorded low soil nematode population densities at UL and ARC. This confirmed the effectiveness of Velum in managing nematode population densities in various crops as observed on potato, tomato and soybean crops (Briedenhann, 2018).

Generally, soil nematode population densities for all sequences at the UL and ARC locations had a similar trend except in Sequence 3. At ARC location, sorghum in the sorghum-potato sequence had a slight significantly lower soil nematode population densities than on *C. africanus* in the *C. africanus*-potato plots. Similarly, when sorghum varieties were included in okra-based cropping systems, soil nematode population densities were reduced by 21% after harvesting the non-host sorghum cultivar (Mweke

et al., 2008). The suppressive effect of sorghum can be attributed to the nematicidal properties of the root exudates released.

Soil nematode population densities in Sequences 1, 2 and 4 were not significantly different at UL and ARC. However, significantly lower soil nematode population densities were recorded in Sequence 3 of each cropping sequence. This showed that the second application of treatment crops or Velum was more effective than the initial application. However, a reduction in soil nematode population densities on potato monoculture plots was unexpected. Such a reduction could be due to the fact that most nematodes had entered the root system and become ‘’trapped’’. Therefore, when the plant was harvested and the soil was sampled, the remaining few non-trapped nematodes were the only ones which could be retrieved from soil samples.

4.4.2 Plant growth variables

Yield and morphological traits: The effect of different plant-parasitic nematodes on growth and development of various crops have been studied over time (McSorley and Dickson, 1995; Russo *et al*., 2007). The different cropping sequences showed significant effects on marketable yield mass at UL and ARC location. The marketable yield mass in the potato-(Velum)-potato and *C. africanus*-potato sequences were significantly higher than that in the potato monoculture and sorghum-potato sequence at UL and ARC locations. The observation was important since the nematode-resistant *C. africanus* was shown to have the potential of serving as a nematode-resistant seedling rootstock in intergeneric

grafting with watermelon (*Citrulus lanatus* (Thunb) Matsum and Nakai) (Liu *et al*., 2015; Pofu, 2012), which also has no *Meloidogyne* species resistant genotypes (Pofu, 2012; Thies *et al*., 2016; Winstead and Riggs, 1959). Accordingly, potato-(Velum)-potato and *C. africanus*-potato sequences were more effective in promoting plant growth than potato monoculture and sorghum-potato under root-knot nematode infested fields.

High marketable yield mass recorded in potato-(Velum)-potato and *C. africanus*-potato sequences could be attributed to the effectiveness of the first treatments in the sequences in reducing the carry-over nematode population densities. In studies by Kimpinski *et al.* (2000) and Wang *et al.* (2007), the inclusion of post-infectional nematode resistant marigold (*Tagetes* species) cultivars in potato-based cropping systems significantly reduced nematode population densities and subsequently increased tuber yield by 14 and 23%, respectively. Similarly, McSorley *et al.* (1994) demonstrated that including different nematode resistant crops in a cropping sequence significantly affected the targeted plant-parasitic nematode population densities, subsequently influencing the overall yield of the successor crops.

Potato monoculture and sorghum-potato plots which had significantly high soil nematode population densities when the potato crop was eventually sown exhibited lower marketable yield mass. Therefore, the observed decrease in marketable yield mass in these cropping sequences could have been due to root-knot nematode damage. Similarly, no tuber yield differences were observed on potato monoculture and pre-

infectional resistant, sorghum-sudangrass-potato cropping sequence established on *M. incognita* infested fields (Crow *et al*., 2001). However, some researchers observed no significant differences in potato tuber yield regardless of the effectiveness of the treatment crop in reducing the carry-over population densities of the target nematode (Crow *et al*., 2000; Larkin and Honeycutt, 2006; Lopez-Lima *et al*., 2013). Other unquantified abiotic and biotic factors could potentially affect tuber yield in a cropping sequence. For example, poor soil structure, soil sickness due to accumulation of toxic allelochemicals released by the preceding crop, low soil fertility and other soil-borne diseases such as blight can reduce tuber yield in different cropping sequences (Koch *et al*., 2020; Sinton *et al*., 2020).

The different cropping sequences showed significant effects on dry biomass at UL. The dry biomass was directly proportional to tuber yield as similarly reported in another study (Iwama, 2008). Plants exhibiting high dry biomass were from cropping sequences with low *Meloidogyne* species RP values and soil nematode population densities. Generally, nematode damage on susceptible plants causes stunted plant growth as observed under potato monoculture in other studies (Korayem *et al.*, 2012; Russo *et al*., 2007)

At both sites, the highest and lowest Mg content on potato leaf tissues were recorded on crops from potato-(Velum)-potato and potato monoculture cropping sequences, respectively. Magnesium content in potato leaf tissue from the potato-(Velum)-potato sequence significantly differed from those in the sorghum-potato and potato monoculture sequences. However, the variable was not significantly different from those in potato leaf

tissues from the *C. africanus*-potato sequence at the UL location. Generally, cropping sequences associated with high RP of *Meloidogyne* species and soil nematode population densities had low Mg content, *vice versa*. Magnesium deficiency negatively affects the activities of enzymes involved in important processes such as respiration and photosynthesis in plants (Guo *et al*., 2016).

Generally, a reduction in most nutrient elements is due to reduced nutrient uptake by the damaged root system of the host plant or reduced nutrient transportation because of deformed vascular tissues or the plant-parasitic nematode feeding on the essential elements (Kirkpatrick *et al*., 1991). For example, fever tea (*Lippia javanica* L.) significantly reduced *M. incognita* soil population density and subsequently increased Mg content in tomato leaf tissues (Mashela *et al*., 2010). However, Blevins *et al*. (1995) reported that Mg content in susceptible soybean leaf tissues increased as soybean cyst (*Heterodera glycines* Ichinohe) nematode inoculum levels increased. In the current study, potato from the sorghum-potato sequence had higher Mg content than *C. africanus*-potato although the former exhibited higher *Meloidogyne* species RP and soil nematode population densities than the later at the ARC location but not at UL location. Locational differences could best explain the differences observed at the ARC and UL locations.

At UL, Na content was significantly high in potato monoculture and differed from the other three cropping sequences which were not significantly different from each other. Similarly, the application of Nemafric-BL phytonematicide reduced root-knot nematode soil

population densities and subsequently improved Na accumulation in green beans (*Phaseolus vulgaris* L.) leaf tissues (Mashela and Pofu, 2017). According to Subbarao *et al*. (2003), plants exhibiting low K content tend to accumulate more Na instead. In this study, potato-(Velum)-potato had the lowest K content. As such, the high Na content recorded in potato-(Velum)-potato could be due to the low K content observed within the potato leaf tissue (Maathuis, 2014). Generally, there is a difference in the redistribution of K and Na in the leaf tissues of nematode infected plants. In one study, plant-parasitic nematode infection reduced K content and increased Na content in citrus leaf tissues (Mashela and Nthangeni, 2002). The differences in the partitioning of K and Na could be attributed to changes in cell permeability or physical damage to root cells which then promotes the uptake of Na over K (Mashela and Nthangeni, 2002).

Potato leaf tissue from *C. africanus*-potato and potato-(Velum)-potato had the highest Ca and P content, whereas intermediate and low Ca and P content were recorded on potato leaf tissues from sorghum-potato and potato monoculture cropping sequences, respectively. Calcium and P are essential elements that influence growth, development and various biochemical functions within the plant (Huber, 1991). Calcium is known to confer resistance to pest and pathogens that attempt to interfere with the normal function of the plant by inhibiting the activity of enzymes released by pest or pathogens and by lignifying the cell wall structure, thereby preventing cell membrane damage or nematode penetration (de Melo Santana-Gomes *et al*., 2013; Marschner, 1997).

Ferraz *et al*. (2010) reported that, plants having high P content had increased plant growth and were less susceptible to nematode damage. At high P content, the plant becomes less susceptible because it exudes small amounts of host-associated chemical cues (de Melo Santana-Gomes *et al*., 2013). Thus, few nematodes are attracted to the root system, thereby decreasing nematode infection incidence (de Melo Santana-Gomes *et al*., 2013; Marschner, 1997; Sato *et al*., 2019). Generally, plants having low Ca and P content are more susceptible to plant-parasitic nematodes (Hurchanik *et al*., 2003) and are greatly associated with poor plant growth (Gomez *et al*., 2017).

The high Ca and P content observed in *C. africanus*-potato and potato-(Velum)-potato could be due to low soil nematode population densities observed in these cropping systems. Generally, Ca and P contents were reduced in leaf tissues of different susceptible plants grown under root-knot nematode infested soils (Abbasi and Hisamuddin, 2014; Carneiro *et al*., 2002; Gomez *et al*., 2017; Goncalves *et al*.,1995; Lobna *et al*., 2017; Mashela and Pofu, 2017; Melakeberhan *et al*., 1987). In contrast, Melakeberhan *et al*. (1987) observed an increase in Ca content as nematode inoculum increased on common bean (*Phaseolus vulgaris* L.) plants. The latter could suggest that a wide range of factors affect nematode × nutrient element interaction in host plants. Basically, the effect of plant-parasitic nematode on plant nutrient status varies depending on a number of factors such as the plant-parasitic nematode species affecting the plant, the duration of infection (Abbasi and Hisamuddin, 2014), the cultivar, age, host-status of the plant, edaphic factors, salinity (Mashela, 2017) and biotic stress factors (Melakeberhan *et al*., 1987). Nevertheless, studies focusing on changes in nutrient

elements content in potato crop cultivated in fields heavily infested with root-knot nematodes have not been well-documented.

4.4.3 Soil health

Generally, poor soil health was observed under potato-(Velum)-potato and potato monoculture as exhibited by low soil organic carbon, microbial diversity and enzyme activity as previously observed in other monoculture cropping systems (Larkin *et al*., 2010, 2012; McDaniel and Grandy, 2016). However, *C. africanus*-potato and sorghum-potato significantly promoted soil health. Therefore, the inclusion of both *C. africanus* and sorghum crops in potato-based cropping system positively influenced soil health compared to potato-potato monocropping. Generally, the inclusion of two or more crops in a cropping sequence resulted in accumulation of soil organic carbon, an increase in soil microbiome diversity and enzyme activity when compared with those in the monocultured fields (Larkin *et al*., 2010, 2012; Liu *et al*., 2006; McDaniel and Grandy, 2016; Xiong *et al*., 2015; Zhang *et al*., 2010). The improvement of soil health under increased crop diversity could be due to the decaying plant residues (root and leaves) or root exudates released into the rhizosphere which comprise simple polysaccharides, amino acids, organic acids, phenolic compounds and higher molecular carbons (Ashworth *et al*., 2017a; Cowgill *et al*., 2002). Thus, as plant diversity increases in a cropping sequence, the rhizosphere becomes enriched by the different organic deposits, thereby improving microbial diversity (Hu *et al*., 2019; Li *et al*., 2016; Qin *et al*., 2017; Wang *et al*., 2012; Zhang *et al*., 2010). For example, *C. africanus* plants produce

cucurbitacin B ($C_{32}H_{46}O_8$), a carbon-rich tetracyclic triterpenoid which occurs in all organs of the plant (Shadung and Mashela, 2016). Although there are no known studies that ascertain the release of cucurbitacin B as a root exudate, the decomposition of root residues or sloughed root cap cells could still be a great source of carbon deposit in the soil. Cucurbitacin B has been associated with the reduction of plant-parasitic nematodes, pathogenic bacteria, and fungi population densities. However, there had been limited information on its overall effect on microbial diversity, particularly within the rhizosphere of actively growing *C. africanus* plants. The current study showed for the first time that soil organic carbon and microbial diversity were higher in *C. africanus*-potato plots than in potato monoculture. Consequently, the *C. africanus*-potato cropping system improved soil health based on the variables measured.

On the other hand, the sugar-rich sorghum crop releases carbon-rich exudates such as sorgoleone ($C_{22}H_{30}O_4$) (Durand *et al*., 2018; Weston *et al*., 2013). Sorgoleone is incessantly released in the rhizosphere throughout the growth cycle of most sorghum varieties and it is readily mineralised by soil microbes existing in different soil types into metabolites that are yet to be characterised (Bertin *et al.,* 2003; Dayan *et al*., 2010; Hennion *et al*., 2019; Weston *et al*., 2013; Weston and Mathesius, 2014). Moreover, the root system of sorghum and *C. africanus* plants are relatively deep, but with extensive top lateral and/or adventitious roots (Collange *et al*., 2011, Navarrete *et al*., 2016, Pofu, 2012). Plants with such root systems can provide adequate water and nutrients for biodiversity, thereby improving soil health (Larkin *et al*., 2010; Navarrete *et al*., 2016).

The low microbial richness and evenness under potato monoculture and potato-(Velum)-potato could be due to limited organic carbon sources for growth of diverse microorganisms (Larkin *et al*., 2011). Roots of different plant species exude different chemicals, which confer interference known as being essential for survival. Qin *et al*. (2017) demonstrated that the accumulation of toxic potato root exudates such as phthalic ($C_8H_6O_4$) and palmitic ($C_{16}H_{32}O_2$) acid under continuous cropping could promote the imbalances of microbial population by favouring growth of certain microbes over others, particularly the low pH-lovers over the high pH-lovers. In potato-(Velum)-potato cropping sequence, lack of microbial diversity could have been aggravated by the application of a toxic chemical synthetic nematicides as previously reported by others (Ashworth *et al*., 2017b; Cowgill *et al*., 2002). Velum ($C_{16}H_{11}ClF_6N_2O$) was originally developed as a fungicide, but was later shown to have nematicidal and insecticidal properties (Oka, 2020), making it a possible broad-spectrum pesticide. Generally, pesticides with broad spectrum are not environment-friendly. Presently, there is limited information with regards to the effect of Velum on non-target organisms such as effective microorganisms (EM) (Oka, 2020). Generally, EM include yeasts, various bacteria and actinomycetes (Mashela *et al*., 2020). In this study, potato monoculture and potato-(Velum)-potato were mapped in different quadrants at both UL and ARC locations. Apparently, the mechanistic effects of these two cropping sequences were significantly different.

Observations in other studies (Ashworth *et al*., 2017a; Bertin *et al*., 2003; Miller *et al*., 2019), demonstrated that potato, maize or soybean monocultures did not compromise microbial diversity. The latter suggested that the effects of cropping sequences on soil

health could be influenced by multiple factors, with the current synthesis focusing on root exudates, root biomass, enzyme activities and environment-specific factors. For instance, *C. africanus*-potato was more effective in improving soil health at the ARC location than at the UL location, whereas the opposite was true for sorghum-potato sequence. The latter could be due to differences in initial microbial inoculum, the prevailing environmental conditions, edaphic factors, plant growth rate, field history and lack of soil homogeneity (Hennion *et al*., 2019; Oberholster *et al*., 2018).

Generally, the reduction in soil organic carbon and microbial diversity subsequently resulted in a decrease in ß-glucosidase, urease and acid phosphatase enzyme activity in potato monoculture and potato-(Velum)-potato cropping sequences. Similarly, low ß-glucosidase (Qin *et al*., 2017), urease (N'Dayegamiye *et al*., 2017; Qin *et al*., 2017) and acid phosphatase (Liu *et al*., 2006) enzyme activities as reported under potato monoculture during the current study, appeared to be a common phenomenon in potato monoculture. Previously (Ashworth *et al*. (2017a), it was shown that the application of pesticides could also reduce enzyme activities. Accumulation of chemical toxins that affect soil pH under monocropping could reduce enzyme activities (Qin *et al*., 2017). Increasing plant diversity in a cropping sequence subsequently increased soil enzyme activity (Steinauer *et al*., 2015; Qin *et al*., 2017). However, the diverse root exudates sometimes neutralise one another as observed for ß-glucosidase activity in cropping sequences involving different crops in another study (Acosta-Martínez *et al*., 2010). The soil organic carbon microbial diversity and enzyme activity can indirectly affect the

nematode feeding type composition in each cropping sequence as observed in a number of studies (Ferris *et al*., 2001; Gupta *et al*., 2019).

Generally, the herbivores were the most dominating nematodes in soil samples collected from all sequences at the UL and ARC locations. The prevalence of was expected since the fields upon which the study was conducted have been continuously used for crop production over a long time. The herbivores were closely followed by bacterivores. As such, bacteria-mediated decomposition pathway was more dominant than fungal-mediated decomposition pathway in most cropping sequences. However, cultivation of potato as the main crop in Sequence 2 and 4 resulted in an upsurge of fungivores. According to Bongers and Bongers (1998), increased fungivore dominance normally occurs when the environment is disturbed or when soil pH is reduced. Furthermore, increased soil disturbances reduced nematode diversity and caused high trophic level nematodes such as omnivores or predators to be the least dominating nematodes (Li *et al*., 2016; Neher, 2001).

Soil samples from the different cropping sequences at UL and ARC were mapped in Quadrant A, C or D of the faunal profile (Ferris *et al*., 2001). Most of the plots with potato-(Velum)-potato, sorghum-potato and *C. africanus*-potato cropping sequences were in Quadrant A, where the soils had moderate to high enrichment, but without structure (Ferris *et al*., 2001; Gupta *et al*., 2019). High EI normally occurs in soils that are predominated by Ba1 bacterivores (Ferris *et al*., 2001). Most agricultural soils are usually mapped into Quadrant A, since high soil disturbance is commonly associated with high

enrichment and low SI, which is exhibited by the absence of K-strategist nematodes (Gupta *et al*., 2019, Habig *et al*., 2018; Malherbe and Marais, 2015; Ugarte *et al*., 2013).

In contrast, potato monoculture plots were mapped in Quadrant D throughout the study, except once in Sequence 3 at the UL location. Hence, these plots were highly resource-depleted and unstable as observed in maize and wheat monocultures (DuPont *et al*., 2014; McDaniel *et al*., 2014). Low enrichment could be due to the unavailability of organic carbon under continuous cropping as previously explained. The inclusion of different crops in cropping sequences increases the variety of food source for soil microorganisms to feed, thereby increasing nutrient enrichment (Gupta *et al*., 2019; Li *et al*., 2016; McDaniel *et al*., 2014; Zhong *et al*., 2016). Since potato monocropping was mapped in Quadrant D, this indicated that continuous cultivation of potato promoted an environment conducive for high fungal activity due to acidic root exudates which promote such activities (Liu *et al*., 2014; Pin-pin *et al*., 2012; Qin *et al*., 2017; Zhong *et al*., 2016).

The faunal profile exhibited in Sequence 4 had a different trend from those observed from Sequence 1 to 3. In Sequence 4, soil from all the cropping sequences were grouped in Quadrant D, except for soil from sorghum-potato cropping sequence which was grouped in Quadrant C. Potato-(Velum)-potato and potato monocropping plots had the lowest stability and enrichment, respectively. At this point, the continuous application of Velum could have disturbed the presence of fungi and free-living nematodes with high coloniser-persister values thereby reducing the structure index. These findings were consistent with

observations made on other pesticide-applied fields (Grabau and Chen, 2016; Gupta *et al*., 2019; Ortiz *et al*., 2016; Zhong *et al*., 2016). However, application of synthetic chemicals had no negative effect on free-living nematodes as observed in another study (Jansen, 2014). The latter suggested that different types of chemicals have different effects on soil health. The low enrichment in potato monocropping was due to limited soil organic carbon and microbial activity as previously observed in the current study.

According to the results in the current study, the soils from sorghum-potato cropping sequences were well structured, but resource-depleted as observed by others (Ferris *et al*., 2001). As such, nematodes with high coloniser-persister values were observed over time in sorghum-potato cropping sequence plots. The improved soil structure by the extensive, fibrous root system and the exudation of sorgoleone (a carbon rich allelochemical) could be the cause for such an observation (Collange *et al*., 2011; Navarrete *et al*., 2016). According to Neher *et al*. (2019), plants that increase organic carbon content and microbial communities could improve occurrence of high trophic level nematodes since food resources would be adequate.

Bongers and Bongers (1998) demonstrated that MI values normally range between 1 and 4. Based on the results, MI of most cropping systems at the UL and ARC locations were below 2 throughout the study, except in Sequence 4, where moderate MI values were observed under sorghum-potato. Low MI values indicated disturbed, enriched, environments, which are generally predominated with r-strategist nematodes (Bongers and Bongers, 1998; Neher and Darby, 2006; Urkmez *et al*., 2014), as confirmed by the

feeding type composition and the faunal profile. The MI in most agricultural soil is usually low, since cultural practices implemented cause high environmental disturbances (Gupta *et al*., 2019; Neher, 2001; Neher and Darby, 2006; Ugarte *et al*., 2013). The moderate MI values observed under sorghum-potato suggested improved soil stability (less disturbances) over time, probably caused by the ability of sorghum cultivars to increase soil organic carbon, microbial diversity and enzyme activity as previously demonstrated in the current study. Hence, the appearance of K strategists was promoted as exhibited by the high SI.

Bongers and Bongers (1998) demonstrated that soil samples having low MI are mostly associated with high PPI, with similar observations in the current study. However, PPI was approximately similar in all plots throughout the current study. Li *et al*. (2016) suggested that the reduction in plant-parasitic nematodes due to different cropping systems could take longer under field conditions. Low CI values dominated most of the treatment plots at UL and ARC. Thus, the decomposition pathways of most soil samples were bacteria dominated, which complimented earlier results from the feeding type assemblages and faunal profile of different cropping sequences investigated in the current study. Generally, bacteria dominated food webs are common in soils where crop rotations are being practiced (Ingham, 2016; Zhong *et al*., 2015). In the current study, extremely high CI values were recorded twice under potato monoculture and once on potato under *C. africanus*-potato, potato-(Velum)-potato and sorghum-potato cropping sequences. As such, the cultivation of potato as the successor crop promoted fungal-dominated decomposition pathways (Neher, 2001).

The Shannon diversity index values were low (≤ 2). Therefore, low nematode species richness was observed on all the plots throughout the study, which suggested that the inclusion of different crops in a potato-based cropping system had no improvement in soil health with regards to nematode diversity. Apparently, the latter could be due to the short experimental periods in the current study as confirmed by others (Larkin *et al*., 2010), caused by soil disturbance due to repeated cultural practices implemented in each cropping system as observed by others (Bongers and Bongers, 1998; Ferris *et al*., 2001). Zhong *et al*. (2015) only observed a change in nematode diversity after 10 years of crop rotation, thereby emphasising the long-term effects of this strategy. Theoretically, cropping sequences where other crops are included normally show high nematode diversity after an extended period (Ingham, 2016; Malherbe and Marais, 2015), but hardly in short-term experiments.

4.5 Synthesis of the study and conclusion

The purpose of the study was to establish the role of different nematode-resistance mechanisms and the efficacy of nematode-resistant crops in potato-based cropping sequences. Based on the results, the investigated cropping sequences had significant effects on population densities of *Meloidogyne* species, plant growth and soil health. Generally, following potato-(Velum)-potato, *C. africanus*-potato sequence was more effective than sorghum-potato and potato monoculture sequences in managing soil population densities of *Meloidogyne* species. The latter manifested through subsequent amelioration of damage to potato crop as shown by high tuber yield, shoot mass and foliar

nutrient elements accumulation at both experimental locations. The ineffectiveness of pre-infectional nematode resistant sorghum crop was attributed to the fact that, sorgoleone might have been quickly hydrolysed into non-toxic substances upon removal of sorghum crop or that the plant-parasitic nematodes might have entered chemiobiosis when sorgoleone was released within the rhizosphere. Alternatively, the quantity and quality of the root exudate might not have been adequate to effectively control the targeted root-knot nematodes due to unidentified factors. The inclusion of post-infectional nematode resistant crops such as *C. africanus* in potato production was a good option for managing root-knot nematode damage under field conditions. In contrast, crops with pre-infectional nematode resistance such as sorghum appeared to protect themselves against nematode damage, but not subsequent susceptible crops such as potatoes. Internationally, studies that compare the effectiveness of different mechanisms of nematode resistance in cropping sequences are limited. In South Africa, although there is ample research on the host status of *C. africanus* and potatoes, there is no documentation of the efficacy of *C. africanus* in managing root-knot nematodes in any cropping sequence. Additionally, not only was the *C. africanus*-potato cropping sequence effective in managing root-knot nematode damage, but it also did not compromise tuber yield when compared to other cropping sequences.

The soil microbial functional diversity and enzyme activity differed among the various treatments and sampling sites. Differences in microbiological indicators of soil health among treatments at different localities were seemingly influenced by the percentage soil organic carbon content. *Cucumis africanus*-potato and sorghum-potato cropping

sequences enhanced soil health much better than potato-monocropping sequences with or without Velum application. Soil organic carbon content, microbial diversity and enzyme activity were promoted when sorghum and *C. africanus* were included in potato-based cropping sequences at UL and ARC locations, respectively. As such both crops were not consistent in promoting soil health across environment.

Based on the nematode community results, MI, CI and SI indices were generally low, whereas PPI generally maintained the same trend across cropping sequences. The latter demonstrated that the soil was highly disturbed when bacteria dominated decomposition pathways. However, the nematode faunal results showed that sorghum-potato cropping sequences improved soil structure at both locations in the long run. Overall, sorghum-potato effectively promoted soil health better than *C. africanus*-potato, potato monoculture and potato-(Velum)-potato cropping sequences, but poorly suppressed population densities of *Meloidogyne* species. Obviously, there is no nematode management strategy that could serve as a panacea to all strategies desired for improved sustainable agriculture. As such, integrated nematode management strategies are recommended to effectively manage population densities of *Meloidogyne* species without compromising soil health in short term potato-based cropping systems. The null hypothesis which suggested that potato monoculture, *C. africanus*-potato and sorghum-potato cropping sequences had no effects on population densities of *Meloidogyne* species, plant growth and soil health was therefore rejected. In the following chapter, the summary of the study, significance of the findings, future recommendations and conclusions were outlined.

CHAPTER 5

SUMMARY AND SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Limpopo Province is one of the major potato producing regions in South Africa. However, little is known about the diversity of nematodes that are associated with potato production. Generally, root-knot nematodes are a known major threat in most potato producing areas in South Africa and all commercially available cultivars are highly susceptible to this nematode genus. The restricted use of chemical nematicides has caused an upsurge in research interest focusing on developing effective and environment-friendly nematode management strategies. The current study investigated the diversity of plant-parasitic nematodes associated with potato production, the efficacy of pre- and post-infectional nematode resistance crops in managing root-knot nematodes in potato-based cropping systems and the influence of each cropping sequence on plant growth and soil health.

The plant parasitic nematode survey conducted in Limpopo Province showed that potato fields were associated with 10 nematode genera. These nematode genera were not simultaneously observed in all the districts. Instead, different nematode species were prevalent in different districts. Nonetheless, *Meloidogyne* species were generally the most prevalent nematodes associated with potato fields, whereas *Tylenchorhynchus* and *Nanidorus* species were the least prevalent.

The current study clearly established the unique role of nematode-resistance mechanisms and the effectiveness of nematode-resistance crops in managing root-knot nematodes in potato-based cropping sequences. Generally, post-infectional nematode resistance in *C. africanus*-potato sequence effectively managed the carry-over *Meloidogyne* species population densities in the soil and reduced root-knot nematode damage to subsequent potato crop. The latter was exhibited by accumulation of high nutrient elements in leaves, higher tuber yield and shoot mass at both locations when compared to those under potato monoculture and under pre-infectional sorghum-potato sequence.

Although low *Meloidogyne* species population densities in the soil were observed when sorghum was cultivated in the sorghum-potato cropping sequences, root-knot nematode population densities increased drastically when the successor crop was cultivated. The latter resulted in lower tuber yield, shoot mass and nutrient elements accumulation, regardless of the location. The observation confirmed the postulation that some pre-infectional nematode resistant plants were having auto-suppressive abilities on *Meloidogyne* species, but were less effective in managing the test carry-over nematodes on the successor crop.

The investigated cropping sequences had significant effect on soil health. The deep extensive root system and high carbon source rhizo-depositions by sorghum-potato and *C. africanus*-potato cropping sequences greatly influenced soil heath. Generally, soil

health improved in cropping sequence where two crops were cultivated compared to when potato monoculture was practiced with or without Velum application. The study demonstrated that Velum application was detrimental to soil health as depicted by low enrichment and structure.

5.2 Significance of findings

A total of eight *(Helicotylenchus*, *Rotylenchulus*, *Paratylenchus*, *Tylenchorhynchus*, *Scutellonema*, *Criconema*, *Nanidorus*, and *Telotylenchus* species) out of the 10 nematode genera *observed* in this study were reported for the first time in association with potato in Limpopo Province, South Africa. Some of the identified genera should be closely monitored to avoid their spread since they have species that are of great economic importance. Findings in the current study demonstrated that, post-infectional nematode resistant *C. africanus* was more effective in managing *Meloidogyne* species carry-over population densities in the soil than pre-infectional nematode resistant sorghum crop when included in sequential crop arrangements as exhibited by reduced yield loss. As such, resource-poor farmers can adopt the use of *C. africanus* plants as a way of managing root-knot nematodes. Internationally, studies investigating the efficacy of locally adapted pre- and post-infectional nematode resistant crops in cropping systems are limited. The current study convincingly demonstrated that crops with pre-infectional nematode resistance are not suitable for use in crop rotations intended for managing *Meloidogyne* species. Furthermore, fewer nematode management strategies' studies focus on the effect of nematode resistant crops on soil health when included in sequential

crop arrangements where the main crop has no resistant genotypes against the yield threatening plant-parasitic nematodes.

5.3 Recommendations

Only soil samples were collected for extraction and identification of plant-parasitic nematodes associated with potato. However, the collection, extraction and identification of nematodes from root samples could give a better picture of the plant-parasitic nematode species which are directly associated with potato in Limpopo Province, South Africa. Additionally, the determination of biological, physical, and chemical properties could provide clarity on the diversity and abundance of nematodes occurring in different districts.

The effectiveness of post infectional nematode resistant *C. africanus* plant in reducing root-knot nematode damage and improving soil health were established, but there is still need for further research on the acceptability of this nematode management strategy by farmers. In nematode management strategies where the allelochemicals produced to repel or kill nematodes have a short life span in the soil such as sorgoleone, the susceptible main crops should be sown soon after harvesting the non-host sorghum crop so as to improve the effectiveness of sorghum in managing nematode population densities.

The pre- and post-infectional nematode resistant sorghum and *C. africanus* plants effectiveness in improving soil health and managing root-knot nematodes could be increased by ploughing back the plants as green manure. Additionally, longer crop sequences could be more effective in managing root-knot nematodes than short cropping systems. Generally, the use of one nematode management strategy can be very risky and less effective under field conditions. As such, integrated nematode management strategies are always highly recommended for the effective management of the targeted plant-parasitic nematodes.

5.4 Conclusions

Meloidogyne species were the most prevalent plant-parasitic nematodes associated with potato in Limpopo Province as reported in other Provinces of South Africa. However, other economically important and less economically important plant-parasitic nematodes were also identified in various districts of Limpopo Province. Therefore, the existence of plant-parasitic nematodes diversity and abundance associated with potato in Limpopo Province, South Africa was confirmed in this current study.

The current study established the role of nematode-resistance mechanisms in crop sequences that involve crops with no root-knot nematode resistance genotypes such as potato. *Cucumis africanus*-potato sequence performed better than sorghum-potato or potato monoculture with regards to improving plant growth, yield and lowering carry-over nematode population densities. Sorghum plants failed to protect the main crop from

nematode damage while it successfully protected itself from nematode damage, but resulted in reduced plant growth and yield of successor potato crop. Continuous cropping of root-knot nematode susceptible crop increased soil population densities of *Meloidogyne* species and caused a reduction in plant growth. However, sorghum-potato outperformed all the other cropping sequences with regards to soil health improvement. In conclusion when the aim is the reduction of nematode population densities, *C. africanus*-potato sequence could be considered, whereas sorghum-potato sequence could be considered where the aim is the improvement of soil health.

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Appendix 3.1 Occurrence of plant-parasitic nematode genera in soil samples from 30 potato fields from Limpopo Province, South Africa.

District	Latitude (S)	Longitude (E)	Sample number	*Nematode genera*									
				Scut	*Heli*	*Para*	*Tyle*	*Melo*	*Telo*	*Roty*	*Cric*	*Nani*	*Prat*
Sekhukhune	24°17'31″	30°21'32″	1	-	-	-	-	-	-	-	-	-	-
	24°24'83″	29°17'10″	2	-	-	-	-	-	-	-	-	-	-
	25°06'27''	29°11'24''	3	-	+	-	-	-	-	-	-	-	-
	25°05'16″	29°11'37″	4	-	-	+	-	+	-	-	-	-	-
	25°01'04″	29°04'24″	5	-	-	-	-	-	-	-	-	-	-
	25°04'24″	29°09'32″	6	-	-	+	-	+	+	-	-	-	-
Capricorn	23°42'36″	29°31'28″	7	-	-	-	-	+	-	+	-	+	-
	23°71'06″	29°63'12″	8	-	-	-	-	-	+	-	-	-	-
	23°86'18″	29°51'64″	9	-	-	-	+	-	-	-	-	-	-
	23°91'67″	29°53'34″	10	-	-	-	-	-	-	-	-	+	-
	22°97'51″	28°83'77″	11	+	-	-	-	-	+	-	-	-	-
	24°21'57''	29°66'29″	12	-	-	-	-	-	-	-	-	-	-

Appendix 3.1 Occurrence of plant-parasitic nematode genera in soil samples from 30 potato fields from Limpopo Province, South Africa (continued).

District	Latitude (S)	Longitude (E)	Sample number	*Nematode genera*									
				Scut	*Heli*	*Para*	*Tyle*	*Melo*	*Telo*	*Roty*	*Cric*	*Nani*	*Prat*
	24°02'51″	29°46'12"	13	-	-	-	-	-	+	-	+	-	-
	24°18'58″	29°29'18"	14	-	-	-	-	-	-	-	+	-	-
	24°01′56″	30°12′56″	16	-	+	-	-	+	-	-	+	-	-
Mopani	24°01′44″	30°20′62″	17	+	-	-	-	+	+	-	+	-	-
	23°58′46″	30°11′84″	18	-	+	-	-	+	-	-	-	-	-
	23°58′17″	30°12′38″	19	+	+	-	-	+	+	-	-	-	-
	24°46′08″	30°28′90″	20	+	+	-	-	+	+	-	+	-	-
	24°49′39″	30°30′14″	21	+	+	-	-	+	-	-	-	-	-
	24°54′06″	30°20′06″	22	-	+	-	-	+	-	-	+	-	-
Waterberg	23°63′15″	27°74′48″	23	-	-	-	-	-	-	-	-	-	-
	23°45'01"	27°51'00"	24	+	+	-	-	-		+	-	-	-

Appendix 3.1 Occurrence of plant-parasitic nematode genera in soil samples from 30 potato fields from Limpopo Province, South Africa (continued).

District	Latitude (S)	Longitude (E)	Sample number	*Nematode genera*									
				Scut	*Heli*	*Para*	*Tyle*	*Melo*	*Telo*	*Roty*	*Cric*	*Nani*	*Prat*
Waterberg	24°53'38"	28°44'06"	25	+	+	-	-	+	-	+	-	-	-
	24°67′44″	28°31′67″	26	+	+	-	-	-	-	+	-	-	+
	23°52′04″	28°40′10″	27	+	+	-	-	-	-	+	+	-	+
	24°41'10"	28°34'16"	28	-	-	-	-	+	-	-	-	-	-
	24°42'10″	28°24'16″	29	+	+	-	-	-	-	+	-	-	-
	23°40'36″	28°11'20″	30	+	+	-	-	+	-	+	-	-	+

Scut = *Scutellonema* species. *Heli* = *Helicotylenchus* species. *Para* = *Paratylenchus* species. *Tyle* = *Tylenchorhynchus* species. *Melo* = *Meloidogyne* species. *Telo* = *Telotylenchus* species. *Roty* = *Rotylenchulus* species. *Cric* = *Criconema* species. *Nani* = *Nanidorus* species and *Prat* = *Pratylenchus* species.

+ = present,

- = not found

Appendix 3.2 Squared cosines of the different genera observed in soil samples collected from potato fields in Limpopo Province, South Africa.

Genera	PC 1	PC 2	PC 3
Scutellonema	**0.897**	0.070	0.03
Helicotylenchus	0.32	**0.682**	0.00
Paratylenchus	0.28	0.03	**0.69**
Tylenchorhynchus	**0.56**	0.33	0.11
Meloidogyne	0.15	**0.53**	0.32
Telotylenchus	**0.93**	0.06	0.01
Rotylenchulus	**0.71**	0.25	0.04
Criconema	**0.87**	0.06	0.071
Nanidorus	**0.56**	0.33	0.11
Cephalobus	0.00	**0.99**	0.01
Pratylenchus	0.76	0.21	0.03

Values in bold correspond for each observation to the principal component (PC) for which the squared cosine is the largest.

Appendix 3.3 Squared cosines of the different districts from which potato fields were sampled in Limpopo Province, South Africa.

District	PC 1	PC 2	PC 3
Mopani	0.00	**0.81**	0.19
Waterberg	**0.88**	0.12	0.01
Capricorn	**0.78**	0.20	0.03
Sekhukhune	0.04	0.10	**0.86**

Values in bold correspond for each observation to the principal component (PC) for which the squared cosine is the largest.

Appendix 4.1 Partitioned sources of variation for plant height (PHT), stem diameter (SDM), chlorophyll content (CLC), marketable yield mass (MYM) and dry biomass (DBM) of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 of potato monoculture, sorghum-potato, *Cucumis africanus*-potato and potato-(Velum)-potato cropping sequences, at the University of Limpopo.

Source of variation	DF	PHT		SDM		CLC		MYM		DBM	
		MSS	TTV (%)[yz]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	5	91.35	6	1.32	27	3.15	1	7277.80	20	2.88	1
Treatment (A)	3	20.58	1[ns]	1.57	32[ns]	3.66	1[ns]	6276.40	18**	9.26	6**
Sequence (B)	1	1532.75	88***	0.09	2[ns]	331.80	96***	19799.00	55**	181.03	91***
A × B	3	7.24	0[ns]	1.02	21[ns]	1.73	1[ns]	994.30	3[ns]	2.77	1[ns]
Error	35	84.21	5	0.89	18	4.35	1	1486.10	4	2.07	1
Total	47	1736.13	100	4.88	100	344.69	100	35833.60	100	198.01	100

[y] Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

DF = degrees of freedom, MSS = Mean sum of squares, TTV = Total treatment variation

[z]***Highly significant at P ≤ 0.01; **Significant ≤ 0.05; [ns]Not significant at P ≤ 0.05.

Appendix 4.2 Partitioned sources of variation for plant height (PHT), stem diameter (SDM), chlorophyll content (CLC), marketable yield mass (MYM) and dry biomass (DBM) of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 of potato monoculture, sorghum-potato, *Cucumis africanus*-potato and potato-(Velum)-potato cropping sequences, at the Agricultural Research Council-Vegetable and Ornamental Plants.

Source of variation	DF	PHT		SDM		CLC		MYM		DBM	
		MSS	TTV (%)[yz]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	5	127.01	11	0.18	1	10.95	9	511.21	5	35.77	9
Treatment (A)	3	31.46	3[ns]	0.26	2[ns]	1.31	1[ns]	6988.06	72**	22.65	6[ns]
Sequence (B)	1	936.85	83**	10.1	87**	104.55	84**	1183.36	12[ns]	307.52	80**
A × B	3	8.69	1[ns]	0.35	3[ns]	2.61	2[ns]	473.66	5[ns]	4.65	1[ns]
Error	35	22.24	2	0.77	6	4.49	4	531.63	6	11.54	3
Total	47	1126.24	100	12.36	100	123.90	100	9687.92	100	382.12	100

[y] Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

DF = degrees of freedom, MSS = Mean sum of squares, TTV = Total treatment variation

[z]***Highly significant at P ≤ 0.01; **Significant P ≤ 0.05; [ns]Not significant at P ≤ 0.05.

Appendix 4.3 Partitioned sources of variation for calcium, potassium, magnesium, phosphorus and sodium in leaf tissues of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 of potato monoculture, sorghum-potato, *Cucumis africanus*-potato and potato-(Velum)-potato cropping sequences at the University of Limpopo.

Source of variation	DF	Ca (%)		K (%)		Mg (%)		P (%)		Na (ppm)	
		MSS	TTV (%)[yz]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	5	0.28	18.63	0.55	12.13	0.13	13.45	9.36E-03	37	1.89E04	5
Treatment (A)	3	0.29	18.79[ns]	1.81	39.82[ns]	0.67	68.01***	7.15E-03	28[ns]	1.57E05	41***
Sequence (B)	1	0.57	37.20[ns]	0.67	14.66[ns]	0.05	4.58[ns]	4.40E-04	2[ns]	1.25E04	3[ns]
A × B	3	0.24	15.44[ns]	0.79	17.35[ns]	0.04	4.43[ns]	4.01E-03	16[ns]	1.62E05	42***
Error	35	0.15	9.95	0.73	16.04	0.09	9.53	4.43E-03	17	3.47E04	9
Total	47	1.52	100	4.56	100	0.99	100	2.54E-02	100	3.85E05	100

[y] Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100

DF = degrees of freedom, MSS = Mean sum of squares, TTV = Total treatment variation

[z]***Highly significant at P ≤ 0.01; **Significant P ≤ 0.05; [ns]Not significant at P ≤ 0.05.

Appendix 4.4 Partitioned sources of variation for calcium, potassium, magnesium, phosphorus and sodium in leaf tissues of the successor potato plants (cv. 'Mondial G3') sown in Sequence 2 and 4 of potato monoculture, sorghum-potato, *Cucumis africanus*-potato and potato-(Velum)-potato cropping sequences at the Agricultural Research Council-Vegetable and Ornamental Plants.

Source of variation	DF	Ca (%)		K (%)		Mg (%)		P (%)		Na (ppm)	
		MSS	TTV (%)[yz]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	5	0.24	12	0.91	25	0.28	40	3.54E-03	30	3.01E04	21
Treatment (A)	3	0.65	33***	1.05	29[ns]	0.25	35**	6.05E-03	50**	2.16E04	15[ns]
Sequence (B)	1	0.85	43***	0.18	5[ns]	0.09	13[ns]	4.5E-05	0[ns]	5.84E04	41[ns]
A × B	3	0.16	8[ns]	0.70	19[ns]	0.03	5[ns]	5.36E-04	5[ns]	4.03E03	3[ns]
Error	35	0.06	3	0.80	22	0.06	8	1.81E-03	15	3.00E04	21
Total	47	1.95	100	3.65	100	0.71	100	1.20E-02	100	1.44E05	100

[y] Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

DF = degrees of freedom, MSS = Mean sum of squares, TTV = Total treatment variation

[z]***Highly significant at P ≤ 0.01; **Significant P≤ 0.05; [ns]Not significant at P ≤ 0.05.