Measuring ecological restoration in conservation agricultural fields in the eastern Free State (South Africa) using nematodes and other soil bioindicators

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ABSTRACT

Conventional agriculture (Conv) has several adverse effects on soil health. For this reason, Conservation agriculture (CA) is implemented as an alternative to aid in restoring soil (ecosystem) health and functioning. However, its restorative impact remains largely unknown due to limited focus on the biological component of agricultural soils. Nematode-based indices (NBIs), namely the Maturity Index 2-5 (MI2-5), nematode faunal analysis, and nematode metabolic footprints (NMFs) were used to measure soil ecosystem health and functioning in three CA farmlands in Vrede and Reitz (eastern Free State, South Africa), before and during the summer growing season. Two reference sites at each study locality included a natural veld area (undisturbed positive reference) and a Conv farmland (disturbed negative reference). Other selected bioindicators included soil respiration (SR), active carbon (AC), and percentage total organic matter (% OM). A further knowledge gap regarding the impact of soil abiotic properties on soil ecosystem health and functioning necessitated the inclusion of soil texture, soil pH and EC, and soil macro and micronutrient analyses. In Vrede, the CA farmlands with a uniform soil texture and cash/cover crop rotation, displayed increased soil ecosystem health and improved soil food web structure as opposed to the Conv reference site (VConv). Farmlands receiving increased inorganic nitrogen (N) inputs presented larger Enrichment (EF) and Bacterivore footprints (BF). The reference site NVV presented the highest values for bioindicators, while the CA farmlands all presented a higher % OM than VConv but displayed varied values of SR and AC. Redundancy analyses (RDAs) indicated the negative correlation of inorganic N with the Structure index (SI) and MI2-5. In Reitz, the CA farmlands with varying soil textures were under cash crop rotation and displayed improved soil ecosystem health and functioning during the second sampling interval, while RConv presented increased soil ecosystem disturbance and decreased soil food web stability during the second sampling interval. A large Herbivore footprint (HF) was observed in most farmlands, especially during the growing season, and in particular for RConv. The impact of clay % on soil ecosystem functioning was confirmed in an RDA that highlighted positive correlations between clay % and bioindicators. Inorganic N was also positively correlated with the bioindicators. Fe also correlated with SI and MI 2-5 measurements prompting the need for further investigations into this trend. Results showed that the studied Vrede CA farmlands were undergoing a process of soil ecosystem restoration, while VConv displayed decreased soil ecosystem functioning. Farmers still utilizing Conv systems are therefore encouraged to consider the benefits of transitioning to CA to increase soil health and agricultural sustainability especially through inorganic N reductions. Despite the implementation of restorative agriculture practices like CA, the effect of soil texture differences on soil ecosystem health and functioning was evident in Reitz and must be considered when measuring soil ecosystem restoration.

Keywords: soil ecosystem recovery; nematode-based indices; bioindicators; soil abiotic properties; conservation agriculture

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CHAPTER 1: INTRODUCTION

1.1 Conventional agriculture in southern Africa

The social welfare of the southern African population and the economic growth of this region is highly dependent on the agricultural industry since it supplies its residents with the means to obtain an income and maintain livelihood (Blein *et al.*, 2013; Thierfelder *et al.*, 2018). These demands are placed on the agricultural industry despite the large climatic pressures it suffers in the form of frequent low rainfall periods and high evapotranspiration rates across the southern parts of the continent (Du Preez *et al.*, 2019). Furthermore, most of the widely used crop production systems have harmful impacts on the environment (Van der Laan *et al.*, 2017). This particularly includes conventional agriculture (Conv) systems, which are typically characterised by a set of practices like mono-cropping, intensive tillage, over-use of synthetic agrochemicals, and inappropriate crop residue management (Sanaullah *et al.*, 2020; Van der Laan *et al.*, 2017).

Conventional agriculture can reduce the ability of soil ecosystems to regulate the occurrence of pests and diseases (Duru *et al.*, 2015; Zhang *et al.*, 2007). An example of this is the aggravation of plant-parasitic nematode infestations, especially root-knot (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.)*,* along with other soil pathogens occurring in Conv systems (Atandi *et al.*, 2017). These systems also promote global warming through the release of greenhouse gasses resulting from intensive tillage practices, which further cause rapid organic matter (OM) decomposition leading to major soil carbon (C) reductions (Sanaullah *et al*., 2020). Furthermore, biodiversity loss, including that of beneficial soil nematodes (Tahat *et al.*, 2020), is an added drawback of mono-cropping and the over-use of inorganic fertilizers and pesticides (Mamabolo *et al.*, 2020). Subsequently, southern Africa's farmlands are rapidly degrading as a result of the rate and manner of recent agricultural intensification (Kopittke *et al.*, 2019). It is estimated that 25 % of South Africa's soils are already severely degraded as a result of anthropogenic activities related to agriculture and forestry (Du Preez & Van Huyssteen, 2020).

1.2 Restoring farmlands under conservation agriculture

There is an urgent need for alternative agricultural approaches that are capable of restoring degraded soils (Palm *et al.*, 2014) and mitigating climate change. Conservation agriculture (CA) is proposed as an approach alternative to Conv, which aims at the sustainable intensification of food production with a lower environmental impact (Swanepoel *et al.*, 2017). The three main principles of CA are 1) minimal or no soil disturbance, 2) permanent soil cover, and 3) crop diversification and/or diversified crop rotations (Brown *et al.*, 2018; FAO, 2014; Hobbs *et al.*, 2008). Additional and complementary to these principles are good agricultural practices that are frequently implemented as part of CA systems. These include the use of cover crops in rotation, livestock integration, alternative nutrient management through organic manures and composts, growing of stress-tolerant crop varieties, as well as the judicious and prudent use of synthetic agrochemicals (Owenya *et al.*, 2011; Rusinamhodzi *et al.*, 2013; Thierfelder *et al.*, 2018; Walters *et al.*, 2016). These principles and practices are ultimately implemented to promote soil health, which allows the restoration of key soil ecosystem functions and services (Van Es & Karlen, 2019).

1.2.1 Soil health and soil ecosystem health

Soil health is defined by Doran and Safley (1997) as "the continued capacity of a soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health". Although soil health is a broad concept that acknowledges the links between the physical, chemical, and biological spheres of soil systems (Yang *et al.*, 2020), the importance of functioning soil ecosystems (i.e. the biological component) in promoting sustainable food production has been increasingly emphasized in recent years (Bünemann *et al.*, 2018; Muñoz-Rojas, 2018; Vink *et al.*, 2020). Healthy soil ecosystems fulfil multiple functions that in turn support ecosystem services including the production of food and fibre, and erosion control through increased soil aggregate stability. Important soil ecosystem functions derived from healthy soils include C sequestration, nutrient cycling, OM decomposition, plant growth regulation, and soil fertility maintenance (Yang *et al.*, 2020).

The implementation of restorative practices, which promote soil ecosystem functioning and service delivery, is vital to increasing global food production and promoting agricultural sustainability (Sánchez-Moreno *et al.*, 2018). Systems under CA present distinct differences in the way that its associated practices impact natural resources compared to the above-mentioned impact of Conv. When implemented correctly, CA can greatly contribute towards increased production and profitability while simultaneously supporting the soil ecosystem (Sanaullah *et al.*, 2020; Seufert *et al.*, 2012). More reliable yields can be delivered by CA farmlands during times of high rainfall variation (Findlater *et al.*, 2019) as CA systems are more climate-resilient (Ghosh *et al.*, 2019b). Furthermore, CA is less dependent on fossil fuel and fertilizer use, therefore increasing economic and environmental sustainability (Findlater *et al.*, 2019; Hobbs, 2007).

1.3 Measuring soil ecosystem restoration

When transitioning from Conv to CA, emphasis must be placed on monitoring the progress of soil ecosystem restoration (Muñoz-Rojas, 2018). Assessing specific indicators of soil ecosystem health and functioning quantifies the performance of the soil ecosystem and measures the benefits that optimally functioning soil ecosystems offer to the environment and producers (Rinot *et al.*, 2019). However, most of the widely used assessment frameworks of soil health, such as the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune *et al.*, 2016) and the Soil Health Tool (also known as the Haney Test) (Haney *et al.*, 2018), utilize limited direct measurements (e.g., soil respiration) of soil ecosystem functioning. Many soil health assessment frameworks, therefore, lack an adequate ecological perspective. Nematodes e.g., were originally considered by the CASH framework due to the extensive use of nematode community information to infer soil ecosystem health and functioning (Bongiorno *et al.*, 2019a; Neher, 2001; Vink *et al.*, 2020). The reasons for not including nematode assessments in the current frameworks are likely because nematode identification is a time-consuming process and requires a high level of expertise, especially when identifying nematodes up to the required taxonomic level. Assessing other bioindicators like microbial community structure and biomass (i.e., phospholipid fatty acid analysis), microbial activity (i.e., enzymes analysis), and microbial diversity (i.e., DNA sequencing) furthermore requires specialised equipment, as well as careful treatment and handling of samples. This is often costly and logistically complex.

Nonetheless, to sufficiently measure both the biotic and abiotic components of soil health remains critical since the capacity of a soil ecosystem to function is a direct product of the various interactions between the soil biological, chemical, and physical components (Thomsen *et al.*, 2012; Yang *et al.*, 2020). Assessing the state of the soil ecosystem in conjunction with predominant abiotic soil factors (e.g., texture, pH, nutrient status, etc.) thus aid in generating valuable information regarding the influence of management practices on soil ecosystem functioning and service delivery (Bünemann *et al.*, 2018; Sanaullah *et al.*, 2020).

1.4 Problem statement

With the adoption of CA, producers aim to farm more sustainably and to promote soil health (Wulanningtyas *et al.*, 2021). To monitor the efficacy of agricultural systems and the progress of restoring soil health, various agricultural laboratories score the health status of soils using mainstream assessment frameworks. However, most of these frameworks place minimal emphasis on the soil's biological component, resulting in limited information on soil ecosystem functioning and its restoration under CA practices. The use of bioindicators which is, e.g., based on the structure of nematode communities, can be used to assess the status of the soil food web and provide information on the ability of soil ecosystems to recover and fulfil functions that support vital ecosystem services. (Bongiorno *et al.*, 2019a; Neher, 2010).

1.5 Aims and objectives

Aims:

This study is aimed at 1) assessing the ecological restoration and functioning of soils under CA compared against pristine, natural veld, and degraded, Conv systems, using nematode-based indices (NBIs) and other biological measurements of soil ecosystem health and 2) identifying key abiotic properties that influence soil ecosystem restoration and functioning in farmlands that transitioned from Conv to CA systems in the eastern Free State (South Africa).

Objectives:

1. Quantify the health and functioning of soil ecosystems under CA by assessing appropriate bioindicators that include NBIs and additional soil biological measurements.
2. Measuring the restoration of soil ecosystems under CA by comparing the status of such systems against a natural veld area (NV; positive reference) and Conv system (negative reference).
3. Identify possible abiotic properties that influence the health and functioning of soil ecosystems by studying the relationships between selected abiotic and biotic parameters.

Hypotheses:

1. The soil ecosystems of farmlands under CA will present increased recovery and functioning after transitioning from Conv practices.
2. Soil chemical and physical properties, which represent the abiotic component of soil health, influence biological processes and therefore soil ecosystem functioning.

1.6 Chapter layout

Chapter 1 Introduction

Chapter 2 Literature review

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Chapter 5 Results and discussion: Reitz

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CHAPTER 2: LITERATURE REVIEW

2

2.1 Agriculture in South Africa

Of the total land area (122 million hectares: ha) in South Africa, 101 million ha are utilized for agricultural purposes and 1.4 million ha for commercial forestry (Du Preez & Van Huyssteen, 2020). The amount of land used for commercial agriculture in 2018 was 46.6 million ha (37.9 %) of South Africa's total land area. This included land for grazing and arable land for crop production of ± 35.6 million ha and ± 7.6 million ha, respectively (StatsSA, 2020). Maize production in South Africa contributes around R9.4 billion annually to the South African economy and is a major contributor to food security in this region (Adisa *et al.*, 2017). During the 2020 growing season, 15.3 million tons (t) of maize was produced on 2.61 million ha in South Africa (CEC, 2020; PRF, 2020). The quantities of cultivated maize and other crops (used in rotation) in South Africa during the 2018/19 and 2019/20 growing seasons are listed in Table 2.1. Data collected by the South African Grain Information Service (SAGIS) indicate that during the grain marketing season of May 2020 to December 2020, an average of 55 793 t of maize was exported weekly.

Table 2.1: The main grain and leguminous crops cultivated in rotation in South Africa and the quantities in which they were produced during the 2018/19 and 2019/20 growing seasons according to the national Crop Estimates Committee (CEC): www.sagis.org.za/CEC (CEC, 2019, 2020).

Crop	**Quantity (t) produced during the 2018/19 growing season**	**Quantity (t) produced during the 2019/20 growing season**
White maize (*Zea mays*)	5 545 000	8 547 500
Yellow maize (*Zea mays*)	5 730 000	6 752 500
Sunflower (*Helianthus annuus*)	678 000	788 500
Soybean (*Glycine max*)	1 170 345	1 245 500
Groundnut (*Vigna subterranea*)	19 455	50 080
Sorghum (*Sorghum bicolor*)	127 000	158 000

Commercial grain and leguminous crop producing farms in South Africa mainly implement conventional agricultural (Conv) practices, which include some form of intensive tillage, resulting in mechanical soil disturbance coupled with high fertilizer and pesticide inputs. This limits the recycling of organic materials (Aune, 2012; Sithole *et al.*, 2016). Soil degradation is furthermore affected by factors like intensive tillage, over-fertilization, high fossil fuel usage, mono-cropping, pesticide application, and overgrazing (Sanaullah *et al*., 2020; Yang *et al*., 2020). These are all Conv practices that also contribute to climate change and global agricultural unsustainability (Sanaullah *et al*., 2020). They accelerate the onset of climate change, especially as a result of the release of large amounts of CO_2 into the atmosphere due to fossil fuel consumption and organic matter (OM) mineralization due to tillage (Arora, 2019; Kopittke *et al.*, 2019; Sarkar *et al.*, 2020).

2.2 Climatic conditions affecting grain crop production

A stable climate is central in supporting continuous global food production (Gomez-Zavaglia *et al.*, 2020; Zinyengere *et al.*, 2013). Production farmlands are adversely affected by climate change, which can lead to increased desertification and nutrient depletion (Arora, 2019). A wide range of crop and ecosystem responses are further anticipated as a result of global climatic instability and general shifts in precipitation patterns and temperatures (Lin, 2011). As a result of climate change, increased pressure from pests and diseases of crops threatens agricultural sustainability (Gomez-Zavaglia *et al.*, 2020; Zayan, 2019), while Conv practices like mono-cropping also intensify pest and disease prevalence (He *et al.*, 2019). Rising temperatures and CO_2 levels heavily influence soil nutrient status, soil moisture, and water availability, which directly affects crop production (Gomez-Zavaglia *et al.*, 2020). In sub-Saharan Africa, producers are compelled to farm in an arid environment threatened by weather extremes such as drought and high seasonal variability (Senyolo *et al.*, 2017; Sithole *et al.*, 2016). The country receives only 50 % of the average global precipitation (Du Preez & Van Huyssteen, 2020), while many of its soils are susceptible to erosion. Water erosion generally causes substantial soil losses in the higher rainfall region of the eastern parts of the country, while wind erosion predominantly causes soil losses in the more arid western parts (Du Preez & Van Huyssteen, 2020). Agriculture in South Africa is furthermore subjected to soil fertility loss that is paired with low rainfall (Swanepoel *et al.*, 2017) where crops must often be produced on highly degraded soils that are depleted of natural resources (Sithole *et al.*, 2016). Many South African soils are considered degraded due to large ecological losses associated with ongoing degenerative practices (Van der Laan *et al.*, 2017).

2.3 The need for increased focus on sustainability

Land-use practices coupled with climatic variability are pressuring the inherent stability of soils (Laker, 2013). Furthermore, soil organic carbon (C) content, which is typically low in South African farmlands, is reduced through mechanical soil disturbance (Du Preez & Van Huyssteen, 2020) that further increases soil erosion rates and contributes to soil degradation (Cardoso *et al.*, 2013). Intensive tillage formed part of the crop production regime of commercial farmers due to mechanization during the industrial revolution of the 1930s (Sithole *et al.*, 2016). During this time, the prolonged reoccurrence of droughts, together with continuous tillage, was responsible for major soil losses of up to 91 million ha in mid-western America (Hobbs, 2007; Hobbs *et al.*, 2008). In addition, Conv is considered to have a large ecological impact resulting from its high rate of mechanization, intensive energy input, and the ongoing depletion of natural resources (Du Preez & Van Huyssteen, 2020; Van der Laan *et al.*, 2017). The salinization of irrigated farmlands, environmental pollution, acidification of soil profiles, soil OM losses, and freshwater aquifer depletion are all further consequences of Conv (Van der Laan *et al.*, 2017). Such occurrences have been projected to cause major yield losses in the future (Sarkar *et al.*, 2020). It is not only Conv but also extensive and unregulated livestock farming that is now impacting up to 2 million ha of land in South Africa by causing subsoil compaction through the unmanaged grazing of maize and wheat (*Triticum* sp.) residues of dryland or irrigation production farmlands (Du Preez & Van Huyssteen, 2020). Furthermore, more than 64 % of South Africa's groundwater is now utilized for irrigation purposes and the country's groundwater utilization for agricultural purposes are drastically increasing (Du Preez & Van Huyssteen, 2020).

To feed a projected population of 9.8 billion by 2050 (UN, 2017), food production must increase by 70 % between 2005 and 2050 (ELD, 2015). However alarming this sounds, there are agricultural approaches, such as organic and conservation agriculture (CA) that may help producers deal with current production challenges especially under adverse climatic conditions (Aune, 2012). There is an evident need for the sustainable management of natural resources and the implementation of adaptive measures that aim to reduce environmental impact while sustaining global food production (Sarkar *et al.*, 2020). Sustainable crop production demands a reduction in the emission of greenhouse gasses, limited nutrient leaching, and decreased pesticide application (Aune, 2012). Therefore, an integrated agricultural system portraying resilience towards a varying climate with a minimized contribution to the onset of climate change is in urgent need (Aune, 2012). So is the conversion of Conv practices to climate-resilient and more sustainable systems (Sarkar *et al.*, 2020).

2.4 Conservation agriculture

Food production through CA practices originated in the 1970s in North America and quickly spread to the temperate and tropical parts of Australia and South America. This occurred in response to the rising concern for continued land degradation caused by continued intensive agriculture (García-Palacios *et al.*, 2019; Kassam *et al.*, 2012). Furthermore, many producers and stakeholders realized that the focus should not only be on the increase of yield, but also on agricultural and environmental sustainability (Sithole *et al.*, 2016). In terms of climate change mitigation, CA is a viable climate-smart option (Senyolo *et al.*, 2017) that is typically associated with lower greenhouse gas emissions due to reduced fossil fuel and fertilizer use (Sanaullah *et al.*, 2020). As a result, CA and sustainable intensification go hand-in-hand, and if CA practices are implemented correctly it can enhance the productivity and reliability of a production system, while simultaneously decreasing agricultural inputs and reducing the effects of climate change (Findlater *et al.*, 2019). Furthermore, CA has been proposed as a means for farmers to better utilize the ecosystems' natural resources (Sithole *et al.*, 2016). The implementation of CA is promoted due to the restorative impact of its practices on soil ecosystem functioning and service delivery (Alikhani *et al.*, 2018; Kassam *et al.*, 2009). It is furthermore able to help sustain key soil ecosystem functions when appropriate crop residue management and minimal soil disturbance are coupled with cover cropping (Jat *et al.*, 2012), especially multiple crop species cultivation, which positively impacts soil ecosystem functioning (Nunes *et al.*, 2018).

2.4.1 Principles of conservation agriculture and their pros, and cons

The various components of a CA system work together to establish a high potential benefit to the farmer and the environment in comparison with that of Conv systems (García-Palacios *et al.*, 2019). The three primary principles upon which CA systems are based are 1) minimum soil disturbance or reduced tillage, 2) permanent soil cover with organic residues, and 3) crop diversification (Hobbs, 2007; Rusinamhodzi, 2015; Sithole *et al.*, 2016). These principles are designed to be interconnected and their implementation can serve to replace lost nutrients and other resources (Lal, 2015).

2.4.1.1 Minimal soil disturbance

Tillage is defined as the process of disturbing the soil through the use of equipment (implements) to incorporate crop residues or amendments into the soil, to prepare a seedbed, and/or to control soil-borne pests and diseases (Hobbs, 2007). Conventional tillage requires high fuel inputs, which increase greenhouse gas emissions, destroys soil pores causing soil structure collapse, oxidates OM, increases soil erosion rates, and breaks down soil aggregate stability (Hobbs, 2007; Rusinamhodzi, 2015). In contrast, the CA principle of minimal soil disturbance, which is applied through the implementation of minimum-tillage or no-tillage practices, results in an agricultural system that causes minimal disturbance in the soil profile (Derpsch *et al*., 2010). Minimum or no-tillage promotes the accumulation and development of a permanent surface mulch that protects the soil surface and provides a buffer against external pressures (e.g., extreme temperatures) (Derpsch *et al.*, 2010). The structural degradation of soils is therefore limited (Senyolo *et al.*, 2017). Reduced tillage practices are further efficient in building soil OM and is known to sequester 0.1-1 t of C per ha per year (Aune, 2012; Lal, 2004). Soils that consequently contain increased amounts of OM can through sequestration reduce greenhouse gas concentrations (Swanepoel *et al.*, 2016). Furthermore, as a food source to organisms occurring in the soil food web, OM determines the abundance of soil microbes, larger soil fauna (Baker *et al.*, 2007), and nematodes as the latter have different feeding requirements (Cheng *et al.*, 2021; Treonis *et al.*, 2010). The accumulation of OM in soils may therefore form microbial decomposition hotspots that favour the occurrence of free-living nematodes (e.g., bacterivores and fungivores) that feed on the resulting abundant microbes (De Goede, 1996; Kitagami *et al.*, 2020; Li *et al.*, 2016). It was furthermore found by McSorley and Gallaher (1991) that high amounts of OM likely caused reductions in certain plant-parasitic nematode numbers under maize and sorghum production (Atandi *et al.*, 2017). The natural enemies of plant-parasitic nematodes may thus be enhanced in a soil ecosystem under minimal soil disturbance (Rahman *et al.*, 2007; Stirling, 1999) causing natural suppression of plant-parasitic nematodes that adversely affect crop production. There is, however, no clear understanding of the direct effect of minimum to no-tillage practices on the population dynamics of plant-parasitic nematodes (Aghnoum & Fizabadi, 2020; Hobbs *et al.*, 2008).

Some drawbacks of no-tillage are discussed by Sithole and Magwaza (2019) and include that farmlands under long term no-tillage practices may present increased soil pH at the soil surface since amendments like fertilizers are not incorporated into the soil profile as in Conv. It may also cause the stratification of soil OM and soil nutrients (Mikha *et al.*, 2013) and might result in increased bulk density at the soil surface (Verhulst *et al.*, 2010). The use of conventional tillage may furthermore be implemented to physically kill and manage high plant-parasitic nematode infestations. The use of this practice is, however, declining in South Africa as CA with the promotion of minimal soil disturbance is increasingly adopted (Jones, 2017).

2.4.1.2 Permanent soil cover

The second principle of CA includes the establishment of a permanent soil cover. Besides the implementation of minimum or no-tillage practices, permanent soil cover can be attained through the cultivation of cover crops as a green mulch or leaving decomposed OM from cash crop or cover crop residues on the soil surface (Derpsch *et al.*, 2010; Sithole *et al.*, 2016). A permanent soil cover decreases run-off, lessening subsequent soil losses (Rusinamhodzi, 2015). It will furthermore effectively mitigate surface compaction by reducing the kinetic energy of raindrops, while also limiting evaporation and conserving soil moisture (Adetunji *et al.*, 2020; Sharma *et al.*, 2018). In terms of weed management, the permanent soil cover and consequent lower surface temperatures might aid in reducing the germinable weed seed bank (Nichols et al., 2015). Permanent soil cover furthermore protects the habitat of soil fauna against the effects of climatic extremes like drastic temperature variations and may result in increased soil OM that serves as a viable food source encouraging the establishment of soil macrofauna (Blanchart *et al.*, 2006; Sithole *et al.*, 2017).

As a permanent soil cover results in lower surface temperatures during summer and higher surface temperatures during winter (Baker *et al.*, 2007), such increases during winter times might cause increases in plant-parasitic nematode numbers (Atandi *et al.*, 2017). In a study by Wu *et al.* (2014), it was found that the greatest plant-parasitic nematode densities were reported under high mean soil temperatures (Atandi *et al.*, 2017). There are also existing concerns regarding the increased use of pesticides, especially broad leaf herbicides in CA systems, because of the lack of tillage that partially assists weed management, and the subsequent dependency on other pesticides (Van der Laan *et al.*, 2017). Such increases in the use of pesticides in CA systems poses a serious threat to environmental, animal and human health (Sanaullah *et al.*, 2020). Pesticide use have been reported to decrease the abundance and diversity of various soil nematodes (Yan *et al.,* 2018) and is a major contributor to global soil degradation (Yang *et al.*, 2020). Economic challenges are furthermore experienced where crop residues can no longer be used as feed for animals or be sold as a direct source of income (Owenya *et al.*, 2011) placing increased financial and management strain on farmers.

2.4.1.3 Crop diversification

Crop diversification in agricultural systems can be obtained through practices like crop rotation, multiple species cover cropping, and intercropping (Vukicevich *et al.*, 2016; Yang *et al.*, 2020) and represents the third principle of CA. When a diversity of crops is grown and rotated it serves to alleviate pest and pathogen pressure, aids in generating biomass, mitigate erosion, and fixes nitrogen (N). It furthermore contributes towards developing an optimal environment for plant roots to maximize their depth and functionality, and aid in obtaining sufficient water and nutrients (Kassam et al., 2009).

However, in areas where all or most of the crops used in a cropping system are susceptible hosts of predominant plant-parasitic nematode species, it might cause a high build-up of such nematodes that have adverse effects on crop yields (Mc Donald *et al.*, 2017; Riekert & Henshaw, 1998). Cultivation of some crops can lead to an increase in plant-parasitic nematode numbers. Sunn hemp (*Crotalaria juncea*), e.g., can increase lesion nematode numbers (Van Biljon *et al.*, 2015). The same crop may, however, in rotation with cash crops, serve to suppress root-knot nematode infestation while also benefiting the following crop (Van Biljon, 2017).

2.4.1.3.1 Traditional crop rotation

The growing of different crops like oat (*Avena sativa*), rye (*Secale cereale*), or wheat can be implemented to increase the uptake of available nitrogen (N) and reduce leaching, while the periodic cultivation of green manure cover crops (e.g., legumes and brassicas) are mainly implemented to supply nutrients to the soil ecosystem (Dabney *et al.*, 2010). The rotation of multiple species of crops and cover crop mixes better supports a functional soil ecosystem and might disrupt the lifecycle of soil-borne pests and diseases by removing their specific hosts for a given time (Yang *et al.*, 2020). Crop rotation furthermore aids in reducing pest and pathogen carryover via crop residues or within the soil (Govaerts *et al.*, 2007) and it may be an effective strategy for soil quality and crop productivity maintenance (Ai *et al.*, 2018). Yield increases of cash crops were previously reported for crops in rotation (Chamberlain *et al.*, 2020; Venter *et al.*, 2016), which was attributed to increased soil ecosystem functioning especially when legumes were used as in rotation (Venter *et al.*, 2016).

2.4.1.3.2 Cover cropping

The cultivation of cover crops may be an effective erosion mitigation strategy that over time increases soil aggregate stability, improving water infiltration (Blanco-Canqui *et al.*, 2013; Sharma *et al.*, 2018). It further provides increased OM input and will not only improve and benefit soil physical, chemical, and biological factors but may ultimately reduce production costs (Adetunji *et al.*, 2020). Soil OM has furthermore been found to be positively correlated with bacterivore and fungivore nematodes (Treonis *et al.*, 2019; Yogaswara *et al.*, 2021) and negatively with plant-parasitic nematodes (Barros *et al.*, 2017; Yogaswara *et al.*, 2021). It was also suggested by Widmer *et al.* (2002) that the practices implemented to increase soil OM content (e.g., crop rotation and cover cropping) can have suppressive effects on plant-parasitic nematode numbers. The roots of cover crops additionally enhance biological tillage, while the surface mulch serves as a food, nutrient, and energy source for soil fauna - which also biologically tills the soil (Hobbs *et al.*, 2008). Subsoil compaction can also be reduced as roots create channels in the subsoil breaking up compaction and increasing water infiltration and soil aeration (Blanco-Canqui *et al.*, 2015). The effective management of cover crop mixes can furthermore contribute to N and C addition to the soil ecosystem, N conservation, optimization of the C:N ratio of crop residues, and improving N availability to the following crop (Dabney *et al.*, 2010). Cover crop mixes can be cultivated to optimize the benefits of cash crop rotation practices (Balkcom *et al.*, 2007). This occurs through the cultivation of cover crops with a high C:N ratio that increases soil OM and subsequently supplies soil nutrients to the next crop (Adetunji *et al*., 2020; Hubbard *et al*., 2013).

The three most common groups of cover crops cultivated together include grasses (Poaceae), legumes (Luguminosae/Fabaceae), and brassicas (Brassicaceae) (Dabney *et al.*, 2010; Sharma *et al.*, 2018). An array of benefits can be obtained from combining the appropriate cover crop mix to address the specific challenges in the production system. The functional traits associated with a specific cover crop will affect the soil ecosystem in which it grows (Bukovsky-Reyes *et al.*, 2019). As a result, cover crops are categorized into groups based on their respective functional traits. The first two groups include two types of grasses, namely C3 and C4 grasses, represented by cotton (*Gossypium* sp.) and maize (*Zea mays*)-like grasses, respectively. The next group consists of legumes that fix N, like cowpea (*Vigna unguiculata*) or hairy vetch (*Vicia villosa*), and the last group consists of non-leguminous forbs such as wheat and sunflower (*Helianthus annuus*) (Sharma *et al.*, 2018; Vukicevich *et al.*, 2016; Yang *et al.*, 2020).

The use of grasses in cover crop mixes has several benefits; grasses are cold tolerant and resistant to decomposition (Dabney *et al.*, 2010; Hubbard *et al.*, 2013), they can furthermore accumulate soil N well during their growing period (Adetunji *et al.*, 2020; Thorup-Kristensen, 2006). Non-legume crops are used in cover crop mixes to help reduce soil erosion and mitigate nitrate leaching (Sharma *et al.*, 2018). In legume-grass cover crop mixes, grasses develop and grow faster, aiding in the prevention of soil erosion. The legumes, in turn, sequester N in the soil that will be available for the grass or following crop (Adetunji *et al.*, 2020; Blanco-Canqui *et al.*, 2015). The immobilization of N in the soil might result from a grass-only cover crop, but mixing legumes with grasses will likely decrease N immobilization (Balkcom *et al.*, 2007). Examples of grasses in cover crop mixes include rye, oat, wheat, barley (*Hordeum vulgare*), and ryegrass (*Lolium perenne*) (Adetunji *et al.*, 2020; Sharma *et al.*, 2018).

The use of non-grasses in cover crop mixes have also been demonstrated. Legumes are typically cultivated as part of cover crop mixes to supply soil surface cover and to help improve soil physiochemical and biological properties (Sharma *et al.*, 2018). They also aid in increasing and maintaining soil fertility, especially in instances where the application of chemical fertilizer was reduced (He *et al.*, 2019). How legumes are managed however determines how and if N will be supplied to the subsequent crop. An example includes legumes that are planted early as they will provide a large amount of biomass and exhibits a greater N contribution to the soil ecosystem (Balkcom *et al.*, 2007). Common legumes used in cover crop mixes include vetch, pea (*Pisum sativum*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*), crimson clover (*Trifolium incarnatum*), sunn hemp, alfalfa (*Medicago sativa*), and others. (Adetunji *et al.*, 2020; Sharma *et al.*, 2018).

Other non-grasses included in cover crop mixes are brassicas. Fast-growing brassicas can take up large quantities of N and their residues can again rapidly mineralize it (Dabney *et al.*, 2010; Hubbard *et al.*, 2013). They can also take up N much faster than other cover crops (Blanco-Canqui *et al.*, 2015; Thorup-Kristensen, 2006). Brassicas like radish (*Raphanus* sp.) have deep taproots that can break up soil compaction layers (Blanco-Canqui *et al.*, 2015). Some brassicas are further known to reduce fungal pathogens in soils (Larkin *et al.*, 2010; Vukicevich *et al.*, 2016) and brassica residues that are incorporated into the soil is increasingly associated with disease suppressive bacteria (Hollister *et al.*, 2013). Brassicas are also known to have biofumigation effects as pesticide volatile compounds are released from decomposing brassicas resulting in the killing of nematodes (Mashela *et al.*, 2017).

Common summer cover crops (SCC) that occur in mixes in South Africa include feed sorghum (*Sorghum* sp*.),* babala (*Pennisetum americanum*), dolichos (*Lablab purpureus*), cowpea, sweet sorghum (*Sorghum* sp*.)*, sunnhemp, and sunflower (GrainSA, 2021). These cover crops are utilized by livestock through specialized grazing during the summer before the formation of seed. Appropriate grazing is, however, encouraged to ensure permanent soil cover. Where possible a winter cover crop (WCC) can be cultivated during the winter months, while a summer cash crop can be annually rotated with a SCC during the summer growing season. Depending on annual winter rainfall, the planting of a WCC mix usually follows. A WCC mix usually includes legumes like red clover (*Trifolium pratense*) or hairy vetch and grasses, cereals, and brassicas like black oat (*Avena strigosa*), fodder radish, barley, turnip (*Brassica rapa* var. *rapa*), and mustard (*Brassica* sp.) (GrainSA, 2021). The grazing of WCC occurs during or after the late winter months into early summer, after which summer cash crops or SCC will be cultivated.

The use of cover crops can, however, present challenges to farmers. The limitations of cover cropping are discussed by Sharma *et al.* (2018) and an example include that some of the species of these crops may be hosts for pests and pathogens during the season when cash crops are not cultivated. As cover crops supply energy to the soil ecosystem, it might potentially increase plant-parasitic nematode numbers (Ferris *et al.*, 2012a). Furthermore, when non-legumes are not removed properly, they may re-emerge during the following growing season posing as competition to the cash crop. The benefits of cover cropping can furthermore take a long time to become evident, and a large initial capital input and changes in labour and machinery are often required.

2.4.1.3.3 Intercropping

The introduction of high crop diversity into especially intercropping systems has been known to cause a 73 % increase in pest and disease suppression (Boudreau, 2013; He *et al.*, 2019). Besides, the implementation of intercropping can increase diversity among soil organisms and enhance ecological intensification; these are benefits that outweighs the possible effects of competition between the different crops (Brooker *et al*., 2015; García-Palacios *et al*., 2019). Furthermore, the pressure of increased plant-parasitic nematode infestation may be alleviated through the cultivation of intercrops with a less favourable (i.e., a poor host or a resistant host) nematode pest host status (Lazarova *et al.*, 2021).

2.4.1.4 Complementary good agricultural practices

Additional to the three main principles are supporting or complementary good agricultural practices, which can be implemented to enhance the functionality of CA systems. Such practices are discussed by Thierfelder *et al.* (2018) and include amongst others: appropriate nutrient management, application of organic manure or compost, the use of stress-tolerant varieties, occasional use of pesticides to relieve pest and weed pressures, and green manure application as an additional groundcover.

The cultivation of cover crops will require an increased capital input (Balkcom *et al.*, 2007) and is therefore frequently implemented together with livestock integration, which provides an economic return, especially in semi-arid regions (Blanco-Canqui *et al.*, 2015). A CA system hosting a livestock component that is dependent on utilizing at least a part of the cover crop as feed adds significant economic value to the agricultural system (Gardner *et al.*, 1991). Furthermore, organic amendments, like cattle manure, are added to the soil through livestock integration (Henneron *et al.*, 2014) advancing soil fertility (Jaleta *et al.*, 2013). It can furthermore increase bacterivore, fungivore, omnivore, and predator numbers in the soil (Li *et al.*, 2010; Pan *et al.*, 2020) while decreasing plant-parasitic nematode numbers (Briar *et al.*, 2007; Pan *et al.*, 2020; Tabarant *et al.*, 2011). Specialized grazing practices that consist of the grazing of multiple areas (e.g., paddocks, grasslands, and farmlands) by the same herd over short grazing periods can be seen as a good agricultural practice. Appropriate livestock management and the above-mentioned CA practices that include cover cropping and crop rotation can strongly complement each other (Rakkar & Blanco-Canqui, 2018). Specialized grazing by ruminants must, however, be implemented with the pre-requisite of leaving 30 % of crop residues as a soil cover (Giller *et al.*, 2009; Jaleta *et al.*, 2013). Specified livestock management or grazing includes both high density and short duration grazing (Chaplot *et al.*, 2016). The effective management of livestock through such grazing practices has previously reversed soil degradation processed by increasing the rate of water infiltration, enhancing the C content of the soil, decreasing the amount of bare soil, and increasing overall soil fertility (Teague & Barnes, 2017).

2.4.2 A typical conservation agricultural system

It was suggested by Thierfelder *et al.* (2018) that the first step towards implementing CA should be to adopt basic good agricultural practices like improved crop varieties and adjusted fertilization. This enables CA to be adopted gradually and in stages, as farmers need to adapt their specific CA system to their local conditions (Thierfelder *et al.*, 2018). Often when CA are not implemented correctly, e.g., when the CA principles are not adjusted to the conditions of the specific farm (Pittelkow *et al.*, 2015), its implementation cannot deliver all the desired benefits (Swanepoel *et al.*, 2017). It is recommended by GrainSA (2021) that South African farmers should implement integrated approaches to soil fertility and acidity management, weed management, pest and disease management, and animal integration with the three principles of CA. In an optimized CA system, the implementation of minimum or no-tillage practices requires the addition of multiple species cover crop cultivation and crop rotation as these are essential practices required to fully integrate the three principles of CA (Derpsch *et al.*, 2010). These three principles are, however, widely adaptable as CA can be practiced on soils with varying physiochemical properties, such as varied soil texture, and can be successful under the cultivation of any crop (Kassam *et al.*, 2019). In Table 2.2 the CA practices of the two study locations are presented. Findlater *et al.* (2019) found that South African farmers are not implementing all three CA principles simultaneously, but each principle is rather *implemented* at different times for different reasons. It was, however, found that no-tillage correlated strongly with the implementation of all three CA principles, possibly indicating that the first step toward CA adoption for South African farmers is the implementation of no-tillage practices. Additional to no-tillage, the number of crops that are rotated by South African farmers mainly depend on the size of their farms (Findlater *et al.*, 2019). The cultivation of cover crops for livestock grazing throughout the year can furthermore be very successful under adequate precipitation (Blanco-Canqui *et al.*, 2015). South African farmers, however, experience varied climatic conditions (Findlater *et al.*, 2019) and must adopt the practice and utilization of cover cropping accordingly (Adetunji *et al.*, 2020).

Table 2.2: The conservation agriculture (CA) systems of the two study localities, Vrede and Reitz, were investigated as part of this project.

	Study locality	
	Vrede	Reitz
Minimum or no soil disturbance	No-tillage	No-tillage
Permanent soil cover	Cover crop cultivation during both the winter (when adequate soil moisture is available) and summer seasons	Limited utilization of cash crop residues by cattle.
Crop diversification	Cover cropping in rotation with summer cash crops like maize or soybean.	Cash crop rotation of soybean, sunflower, and maize. Winter wheat is also cultivated when adequate soil moisture is available.

2.4.3 Adoption of conservation agriculture

In 1973/74, CA was practised on only 2,8 million ha globally. In 2008/09 the adoption rate increased to 106 million ha and in 2013/14 to 157 million ha globally. Finally, in 2015/16, the global adoption rate was estimated at 180 million ha (Kassam *et al.*, 2019). In a study by Findlater *et al.* (2019), it was found that only 14 % of the commercial grain farmers in South Africa have adopted CA by implementing all three principles effectively. Despite this low CA adoption rate, the individual CA principles were implemented to a larger degree with adoption rates greater than 40 % for the individual principles. Minimum soil disturbance and permanent soil cover was reportedly adopted by two-thirds of the studied farmers. The implementation of no-till practices was rather determined by factors like rainfall of the various regions and farm size. It was, therefore, suggested that no-tillage may be an entry-level practice to ultimately adopt CA entirely. Senyolo *et al.* (2017), however, reported that the farm-based changes associated with the management intensity of CA may hinder its implementation in South Africa. In 2008–2009, 368,000 ha (2.4 %) were under CA in South Africa (Derpsch *et al.*, 2010; Van der Laan *et al.*, 2017). Currently, still, less than 5 % of South Africa's arable land is under CA, with the remaining land area being under Conv that will likely cause large-scale soil degradation (Du Preez & Van Huyssteen, 2020). The implementation of CA must therefore be advocated in South Africa as an alternative practice that can mitigate soil degradation and restore soil health in agricultural farmlands (Du Preez & Van Huyssteen, 2020; Sithole *et al.*, 2016)

2.5 Soil health

2.5.1 Defining soil health

Soil health is defined as: "The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health" (Doran & Safley, 1997). This definition of soil health is used by various authors, including Bünemann *et al.* (2018) and Cardoso *et al.* (2013). The complex nature of belowground interactions and functioning of the soil ecosystem is thoroughly considered within this definition as well as how this links to the delivery of soil ecosystem services (Bünemann *et al.*, 2018). Agricultural advancement and the development of various cropping systems with different environmental impacts furthermore caused our understanding of soil health to consequently evolve (Yang *et al.*, 2020).

Soil health is further defined as the ability of a soil to function and deliver ecosystem services (Van Es & Karlen, 2019). Or as suggested by Cardoso *et al.* (2013), as "The ecological equilibrium and the functionality of a soil and its capacity to maintain a well-balanced ecosystem with high biodiversity above and below the surface, and productivity." To furthermore differentiate between the terms soil quality and soil health, Pankhurst *et al.* (1997) suggested soil quality be defined as a soil's capacity to meet specific human needs and soil health being "soil's continued capacity to maintain its functions." Nowadays the terms soil quality and soil health are used interchangeably (Brevik *et al.*, 2017; Muñoz-Rojas, 2018; Yang *et al.*, 2020). In essence, soil health comprises a holistic perspective and serves as an umbrella term that includes the many complex interactions that form part of soil processes (Karlen *et al.*, 1997).

2.5.2 Measuring soil health

There is currently no way of directly measuring soil health. However, the product(s) of effective soil ecosystem functioning can be measured as it is reflected through specific attributes that serve as accurate indicators (Lal, 2016). Soil attributes that respond rapidly to environmental changes induced by natural or anthropogenic influences (Cardoso *et al.*, 2013), which is strongly associated with soil processes (Yang *et al.*, 2020) and accurately reflect soil ecosystem functioning, are considered good indicators of soil health (Bünemann *et al.*, 2018). Research suggests that evaluating changes in soil physical, chemical, and biological properties may be the best means to assess and monitor soil health (Doran & Safley, 1997; Moebius-Clune *et al.*, 2016; Verhulst *et al.*, 2010). Some of the major frameworks used in South Africa and other parts of the world (e.g., the United States of America) include Cornell's Comprehensive Assessment of Soil Health (CASH) framework (Moebius-Clune *et al.*, 2016) and the Haney Soil Health Tool (Haney *et al.*, 2018). The widely used CASH framework considers soil chemical indicators like pH, soil macro and micronutrients status, as well as soil physical indicators including soil texture, bulk density, surface, and sub-surface hardness, and available water capacity. Soil biological indicators furthermore include the measurement of OM content, soil microbial respiration, soil proteins, active C, among others. (Moebius-Clune *et al.*, 2016).

2.5.3 Soil health and ecosystem functions and services

Practices based on the principles of CA are designed to restore major soil functions like C transformation, nutrient cycling, soil structure maintenance, and biological pest control (Du Preez *et al.*, 2018b; Kibblewhite *et al.*, 2008) leading to an increased soil health status (Sanaullah *et al.*, 2020). Benefits of the functioning of a healthy soil, which supports biological activity, include the promotion of environmental quality and the enhancement of plant, animal, and human health (Du Preez & Van Huyssteen, 2020). Biological soil processes are responsible for C cycling in the soil ecosystem and the enablement of continued photosynthesis, which is a driver for nutrient mineralization (Cardoso *et al.*, 2013). The billions of microscopic organisms that are responsible for nutrient mineralization and making it available to plants must in turn be sustained through sufficient C compounds, which are primarily supplied by a healthy soil as a result of its functioning (Pettit, 2004).

Soil ecosystem services relate to the well-being of humans and include the provision of food, fibre, freshwater, pest and disease control, climate regulation, erosion mitigation, soil formation, nutrient cycling, pollination, provision of habitat, and the addition of general aesthetic value (Adhikari & Hartemink, 2016; Duru *et al.*, 2015; Stavi *et al.*, 2016). Soil ecosystem functioning, in turn, serves to support the delivery of the above-mentioned ecosystem services (Adhikari & Hartemink, 2016;

Hannam & Boer, 2004). Yang *et al.* (2018) defined ecosystem functions as "the processes that link abiotic and biotic components in ecosystems, e.g., fluxes of material and energy between soil biota and plants affecting plant productivity".

Effectively, functioning soil ecosystems result in the establishment and maintenance of an environment in which plants, animals, and humans can thrive (Haslmayr *et al.*, 2016; Stavi *et al.*, 2016). Organisms in different functional groups co-exist in the soil ecosystem to promote the delivery of ecosystem functions (Kibblewhite *et al.*, 2008). These occur as mesofauna like nematodes, protozoans, and microarthropods that feed on plant substrates and microbes (e.g., fungi and bacteria), and decomposed residues that are in turn consumed by soil macrofauna like earthworms and macro arthropods (Hendrix *et al.*, 1986; Kibblewhite *et al.*, 2008; Petersen & Luxton, 1982). A graphic representation of the soil food web is given in Figure 2.1.

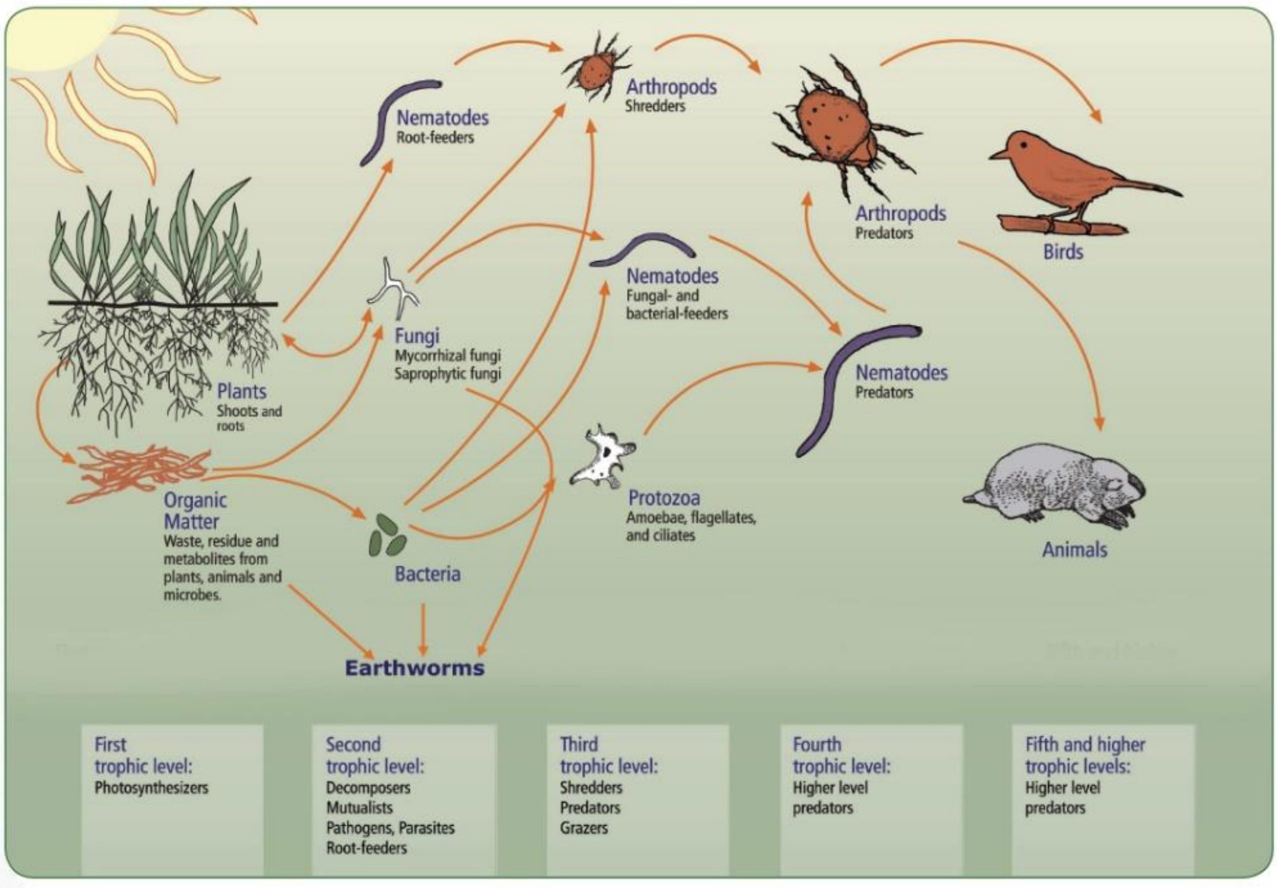


Figure 2.1: The soil food web illustrating energy and matter flow through multiple trophic levels, each indicating their inhabiting organisms/biota, in the soil ecosystem (Moebius-Clune *et al.*, 2016).

The initial supply of energy to the soil food web is in the form of OM through crop growth (cover cropping) or organic amendments (Ferris *et al.*, 2012a; Zhang *et al.*, 2017). Energy and matter flow through the soil ecosystem via all inhabiting organisms (De Angelis, 2016). The supply of soil OM to the soil food web directly affects the state of soil chemical, biological, and physical properties. If soil OM is adversely affected the functionality of the soil ecosystem is reduced (Alikhani *et al.*, 2018; Sithole *et al.*, 2016). Furthermore, the specific feeding habits of different soil biota plays a fundamental role in the flow of energy through the soil food web as organisms with similar feeding habits or trophic roles are grouped into assemblages (De Angelis, 2016; Kibblewhite *et al.*, 2008). The measurable condition of these assemblages is considered a direct expression of the stability of the soil ecosystem (Du Preez *et al.*, 2018b).

Degradation of the soil ecosystem is evident after decades of continuous intensified, degenerative practices (Van der Laan et al., 2017). Mechanical soil disturbance increased soil erosion rates and caused major declines in soil OM content (Cardoso *et al*., 2013; Sithole *et al*., 2016). As losses in soil OM intensifies, the soil ecosystem becomes increasingly dependent on external inputs to support crop growth (Cardoso *et al*., 2013; Kopittke *et al*., 2019). Practices associated with increased external inputs (referring to Conv practices) are currently causing increased soil degradation (Du Preez & Van Huyssteen, 2020). Soil ecosystem restoration should therefore not just entail soil ecosystem recovery to the point of supporting crop growth but must be aimed at re-establishing and enhancing ecosystem functions and services (Costantini *et al.*, 2016; Muñoz-Rojas, 2018). Soil ecosystem functioning is further largely dependent on the occurrence, maintenance, and activity of soil biota, which subsequently depends on plant growth that adds C to the soil ecosystem (Vezzani *et al.*, 2018).

2.6 Measuring soil ecosystem functioning

With the integration of the CA principles into an agricultural production system, it is important to define clear implementation goals to aid the development of appropriate assessment and monitoring strategies, especially when working towards restoring soil ecosystem functioning (Muñoz-Rojas, 2018). The assessment and monitoring of important soil factors, which affect soil ecosystem productivity like hydraulic properties, pollution levels, soil nutrient status, soil organic C, and soil biodiversity, and others, are pre-eminent to reach global sustainable development goals (Toth *et al.*, 2018). Monitoring the state of soils and the frequency and degree of changes in the soil ecosystem are necessary to inform decision and policy making and subsequently require the accurate identification of key indicators of soil health and ecosystem functioning to be measured (Muñoz-Rojas, 2018; Seaton *et al.*, 2020).

Modern-day cropping systems were initially designed to produce maximum yield, but due to the continued deterioration of the soil ecosystem's functioning over time their sustainability is now in question (Fargione *et al.*, 2018). Therefore, the measuring and monitoring of soil health and soil ecosystem functioning became an important factor in agricultural management. Also, in the light of recent climatic challenges and despite varying climatic conditions, soils with an increased soil health status may still be able to produce an increased crop yield (Congreves *et al.*, 2015). To asses soil ecosystem restoration, researchers must have a thorough understanding of the various abiotic and biotic factors, as well as the broad range of environmental attributes, that are at play in soil ecosystems (Muñoz-Rojas, 2018). As displayed in Figure 2.2, there is a direct relationship between soil biological, chemical, and physical indicators and soil ecosystem functioning. Improvements in soil properties will subsequently enhance the functioning of the soil ecosystem and monitoring the appropriate indicators will therefore be adequate to represent the state of the soil ecosystem in terms of soil ecosystem functioning (Muñoz-Rojas, 2018).

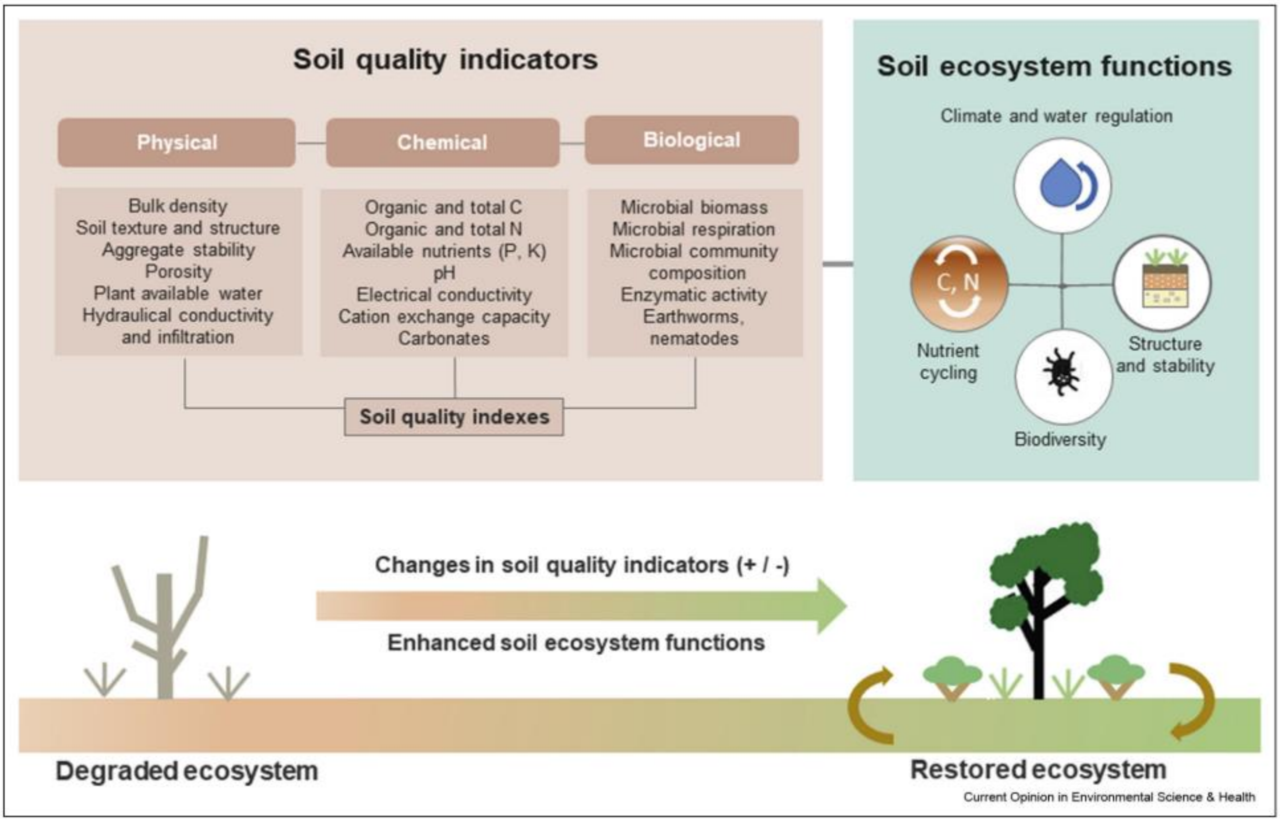


Figure 2.2: A representation of the important relationship between different soil health indicators and soil ecosystem functions, adapted from Muñoz-Rojas (2018).

It is only in recent times that soil biology and soil biological processes became increasingly used as accurate bioindicators of soil health and soil ecosystem health and can in a sense be seen as neglected (Moebius-Clune *et al.*, 2016; Yang *et al.*, 2018). The soil ecosystem is no longer only recognized for the single output of yield or crop growth but is seen and measured as a multifunctional environment dependent on a constant equilibrium (Bünemann *et al.*, 2018; Doran & Safley, 1997). The biological, chemical, and physical properties of soil portray a soil ecosystems' capacity to function.

2.6.1 Indicators of soil ecosystem functioning

2.6.1.1 Chemical indicators

Soil nutrient unavailability is detrimental to plant health. Subsequently, the soil nutrient status determines the overall health of cultivated crops (Moebius-Clune *et al.*, 2016). The macronutrients that plants require to optimally grow include N, phosphorus (P), and potassium (K). These are commonly present in insufficient amounts in soils (Moebius-Clune *et al.*, 2016). Fertilizer containing macronutrients, and often also secondary nutrients like calcium (Ca), Mg, and sulphur (S) - and micronutrients - iron (Fe), zinc (Zn), boron (B), copper (Cu), and others, are therefore applied to soils (Moebius-Clune *et al.*, 2016). Managing soil nutrient status helps support plant growth, but over-fertilization can have adverse effects on the soil ecosystem through modification of the soil's chemical and physical structure in the long-term causing possible ecosystem degradation (Zhang *et al.*, 2020). An increased focus on the essential plant macronutrient, P, is also important since its deficiency can easily induce large crop yield losses (Cardoso *et al.*, 2013). In conjunction with primary and secondary macronutrient measurements, soil pH cannot be omitted as it is a key indicator of soil chemical properties that determines nutrient availability and affects the microbial community structure (Cardoso *et al.*, 2013; Moebius-Clune *et al.*, 2016). Assessing soil pH, therefore, helps researchers to predict the availability of nutrients to the soil ecosystem in farmlands (Cardoso *et al.*, 2013).

2.6.1.2 Physical indicators

Soil physical properties can be seen as drivers of various soil ecological processes (Erktan *et al.*, 2020). Soil physical indicators such as soil texture are stable for a long time and are measured to serve as a basis for interpreting results from other indicators (Moebius-Clune *et al.*, 2016). Soil texture can directly affect the breakdown of soil OM in the soil ecosystem, while also affecting soil microbial biomass over time (Acosta-Martínez *et al.*, 2003; Li *et al.*, 2018). Soil bulk density, the soils' dry weight per unit volume of soil (Moebius-Clune *et al.*, 2016), is also influenced by soil texture and structure and is related to soil compaction, which in turn affects water and oxygen supply to plants and organisms (Schoenholtz *et al.*, 2000). Soil texture further determines soil aeration, its water holding capacity, and also root system topography, which in turn affects the soil biotic community structure (Quist *et al.*, 2019; Ruamps *et al.*, 2011).

2.6.1.3 Biological indicators of soil health

The soil biological component is a good soil health indicator that is often considered more informative than soil physiochemical properties as the latter takes much longer to respond to environmental changes (Cardoso *et al.*, 2013). Soil OM content is a major biological component affecting a wide spectrum of soil processes (Moebius-Clune *et al.*, 2016) such as soil aggregation, compaction, crusting, soil ecosystem energy fluxes, and soil microbial activity (Blanco-Canqui *et al.*, 2013). It is primarily responsible for energy provision to soil microorganisms affecting the functioning of the microbiome (Lal, 2016). Measurements of the soil OM content of soils must therefore be included when assessing soil health. Another biological indicator of soil health, which is more sensitive than soil OM content, is active or labile C (AC). This form of C in the soil ecosystem is utilized as a food and energy source by soil biota, therefore, supporting the maintenance of a healthy soil food web (Saini *et al.*, 2021). Due to its utilisation by soil biota and labile form, AC is representative of changes in soil ecosystem processes (Bünemann *et al.*, 2018; Gregorich *et al.*, 1994) and is therefore an accurate indicator of soil health (Bongiorno *et al.*, 2019b). Soil respiration (SR) furthermore represents the metabolic activity of the soil microbial community measured as the amount of CO_2 respired (Lloyd & Taylor, 1994). It is considered the largest C flux occurring from soil ecosystems into the atmosphere (Chen *et al.*, 2018) balancing terrestrial energy flow and nutrient cycling (Chen & Chen, 2019; Luo & Zhou, 2006). Measuring SR as an indicator of the status of biological activity in the soil ecosystem (Ebrahimi *et al.*, 2019) is therefore considered an important biological indicator. Furthermore, when assessing soil food web stability and ecosystem functioning, it is best to select appropriate representative groups of organisms occurring throughout the soil ecosystem. One such group is nematodes, which will be discussed in the next Section.

2.7 Using nematodes as bioindicators of soil ecosystem health

Nematodes are considered the most abundant of sensitive soil organisms that respond rapidly to environmental changes as a result of their broad range of food sources. They consequently occupy key positions in the soil food web allowing analysis of their community structure as a measure of soil ecosystem disturbance (Bongers & Ferris, 1999; Ferris *et al.*, 2001). Nematodes are unsegmented pseudocoelomates that are classified into the phylum Nematoda and which occurs as free-living/beneficial and plant, and animal (vertebrates and invertebrates) or human parasites (Eyualem *et al.*, 2007). As mentioned above, nematodes function in multiple trophic levels in the soil food web (Figure 2.1) and are among the most miscellaneous of soil biota (Cheng *et al.*, 2021; Sánchez-Moreno *et al.*, 2018; Yeates, 2003). Nematodes are found in marine, freshwater, and terrestrial environments (Eyualem *et al.*, 2007; Hodda *et al.*, 2009) and occupy a central position in the soil food web as a result of their close functional and trophic interactions with the soil microbial community (Cheng *et al.*, 2021; Li *et al.*, 2016; Yeates *et al.*, 1993). Soil nematodes can detect a food source within 50 cm and can move between 0.03 cm and 0.15 cm per hour (Dusenbery, 1983; Erktan *et al.*, 2020; Rasmann *et al.*, 2012).

These organisms have been labelled as plant parasites or herbivores considering the major economic losses occurring when their numbers exceed damage threshold levels in farmlands under continuous crop production. It is, however, estimated that only 10 % of nematodes cause damage to plants (Moura & Franzener, 2017). As opposed to plant-parasitic nematodes, beneficial nematodes feeding on fungi, bacteria, and other soil organisms have a positive effect on soil food web functioning as they decompose OM, cycle nutrients, and improve soil fertility (Buchan *et al.*, 2013; Nguyen *et al.*, 2020). As shown in Figure 2.3 a-e, nematodes are categorized as bacterivores, herbivores/plant-parasites, fungivores, omnivores, and predators (Liu *et al.*, 2019; Yeates *et al.*, 1993).

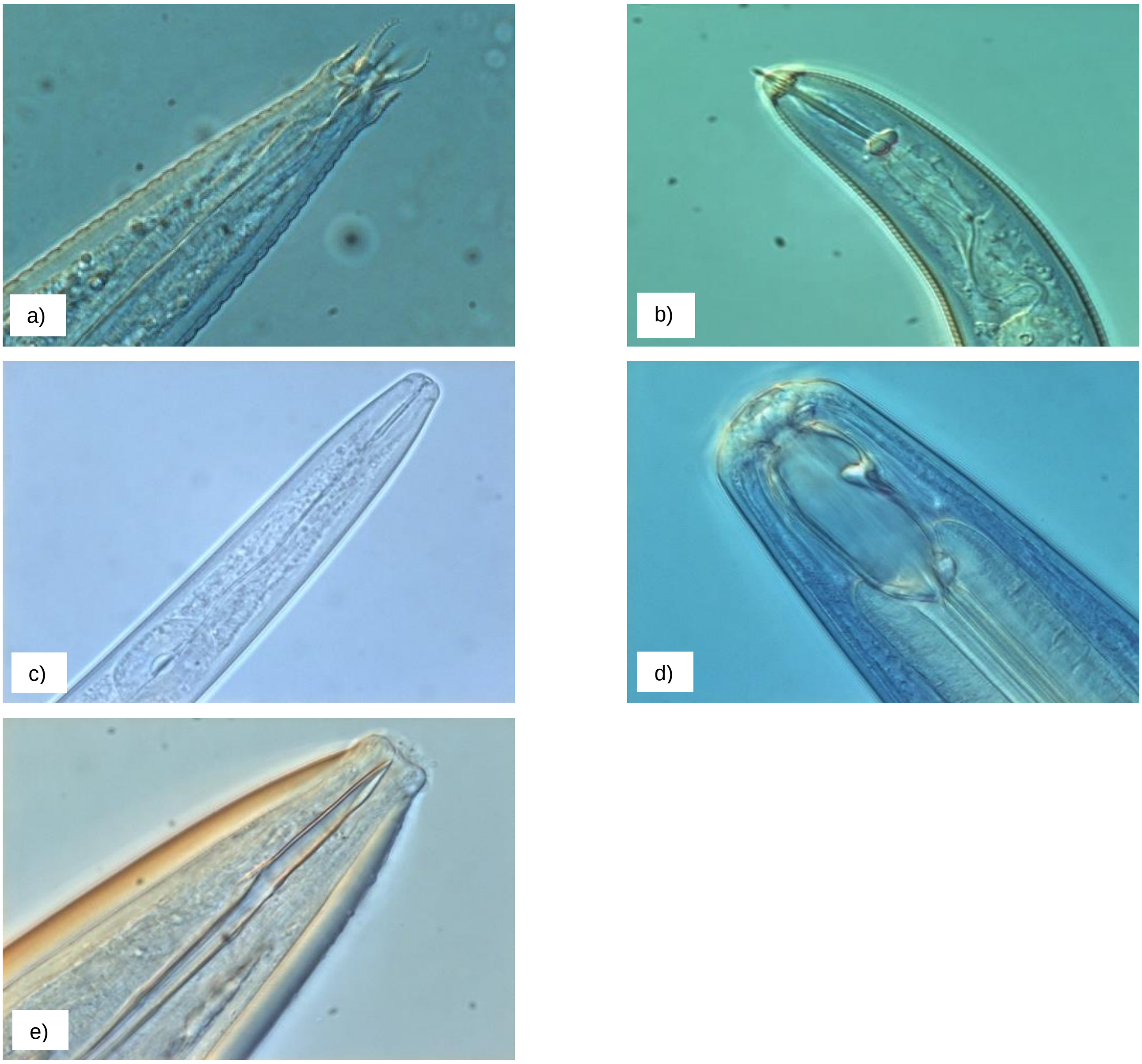


Figure 2.3: a-e) Five nematode trophic groups a) Bacterivore, b) Herbivore/Plant parasite, c) Fungivore, d) Predator, and e) Omnivore (Photos: sourced from Wageningen University & Research (Wageningen, 2018)).

Nematodes are present in both the soil and surface litter (Cheng *et al.*, 2021) and their abundance is partly determined by the presence of respective food sources (Cheng *et al.*, 2021; Treonis *et al.*, 2010). These organisms are very responsive to changes in food availability, as well as physical and/or chemical disturbance, and are therefore used as ecological bioindicators (Bongers & Ferris, 1999; Carneiro *et al.*, 2019; Du Preez *et al.*, 2018b; Sánchez-Moreno *et al.*, 2018).

Nematode community structure has therefore been used as a major indicator in soil health assessments (Ney *et al.*, 2019; Ritz *et al.*, 2009). The Nematode Indicator Joint Analysis (NINJA) online tool was developed by Sieriebriennikov et al. (2014) and is frequently used to calculate nematode-based indices (NBIs). The calculation of NBIs is mostly based on the functional guild classification of the nematode community according to their life-history characteristics and trophic group (i.e., herbivore, bacterivore, fungivore, omnivore, or predator). At family and sometimes genus level, nematodes are placed into one of five classes as part of a colonizer-persister (c-p) series (Bongers & Ferris, 1999). Nematodes categorized as c-p1 have short life cycles with population dynamics that are rapidly responsive to changes in the environment. They are known as enrichment opportunists and are commonly found in disturbed environments. Nematodes categorized as c-p5, in turn, are intolerant of environmental disturbances and with prolonged life cycles, they inhabit more stable and mature ecosystems. These nematodes are known as extreme persisters. E.g, nematodes from the family Rhabditidae are classified Ba1 (i.e., bacterivore with a c-p1 value), while individuals from the family Monochidae are classified as Ca4 (i.e., predator with a cp-4 value).

The proportions in which these functional guilds occur can be representative of various soil food web attributes and encouraged the development of an enrichment (EI) and structure (SI) indices which subsequently correlate with nutrient enrichment within the soil ecosystem and soil ecosystem maturity, respectively (Ferris *et al.*, 2001; Reichel *et al.*, 2017). The faunal profile (Figure 2.4) is a visual representation of the state of the soil food web (Du Preez *et al.*, 2018b; Ferris *et al.*, 2001), as each quadrat represents the respective conditions of the soil food web as inferred by indicative nematode taxa. The EI increases in the soil food web as a result of increased availability of resources such as organism mortality (Ferris *et al.*, 2001; Odum, 1985) or management practices like organic amendments (Pan *et al.*, 2020). The SI is typically higher in environments that are actively recovering from stress presenting increased soil food web complexity (Ferris *et al.*, 2001; Lazcano *et al.*, 2021).

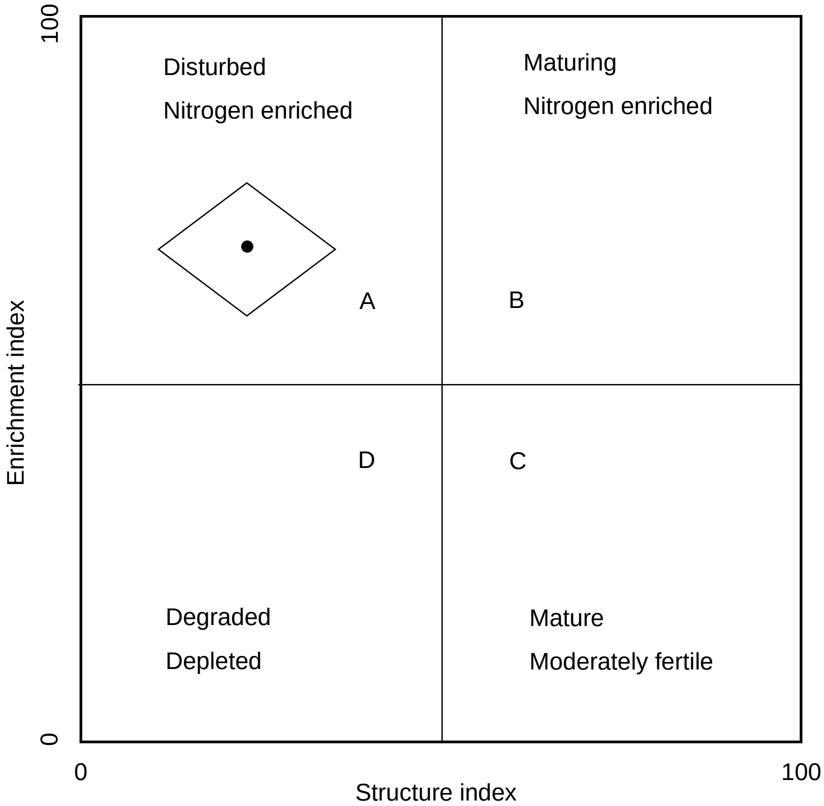


Figure 2.4: A faunal analysis used to indicate the status of the soil food web by means of plotting an environment (e.g., farmland) in one of four quadrats (A, B, C and D) according to the abundance and diversity of structure and enrichment nematodes. The faunal analysis also contains a metabolic footprint (rombus) in Quadrat A that represents the enrichment and structure footprints on its vertical and horizontal axes respectively (Illustration: Ané Loggenberg, NWU).

The utilization of C within the soil food web prompted the development of an index known as nematode metabolic footprints (NMFs), which quantifies the magnitude of ecosystem functions and services delivered by nematodes and other soil food web components (Ferris, 2010; Ferris *et al.*, 2012a; Zhang *et al.*, 2015b). The footprints of the various nematode tropic groups e.g., Bacterivore footprint (BF) or Fungivore footprint (FF), represents the flow of C through their respective trophic channels. A representation of the NMF is presented on the faunal analysis (Figure 2.4). The centre point is the mean value of the Structure index (SI) and Enrichment Index (EI) and indicates the quadrat in which the soil ecosystem plots, while the vertical and horizontal axes of the rhombus represent the Enrichment (EF) and Structure (SF) footprints, respectively (Ferris, 2010; Kou *et al.*, 2020).

2.8 Conclusion

Literature indicates that resilient soil ecosystems with sustained ecosystem functioning through the implementation of logical, affordable, and sustainable agricultural management practices are urgently needed for agricultural sustainability (Lin, 2011). To promote the restoration of soil ecosystem functioning in agricultural farmlands, the development of a means to holistically assess and monitor its progress is a requisite. It is fairly unknown how the implementation of agricultural practices as rehabilitation techniques affects the restoration of soil ecosystem functioning over time (Li *et al.*, 2016). This is especially applicable to South African farmlands since the adoption of management practices to improve soil health particularly aimed at using an integrative set of biological indicators (Yang *et al.*, 2018) is slow and limited research has been done in this regard to date (Findlater *et al.*, 2019).

Implementation of monitoring approaches where ecosystem functioning is considered and correctly interpreted is, however, uncommon (Bünemann *et al*., 2018). This occurs especially in the context of agricultural management. Considering soil ecosystems in farmlands under CA will provide valuable insight into utilizing profitable yet sustainable agricultural management to increase global food security. It provides a research opportunity focused on soil ecosystem recovery under specific agricultural practices, like CA and Conv where bioindicators can be utilized in an integrative way as an accurate measure. A further research gap exists as a need to generate knowledge and develop an understanding of the influence of the abiotic soil component on different soil biological processes (Birkhofer *et al.*, 2012; Salamun *et al.*, 2017; Vincent *et al.*, 2018) as there is a need to identify the possible abiotic drivers of soil biological processes. Increased knowledge of such relationships will assist researchers and farmers to comprehend and predict the functioning of soil ecosystems of farmlands based on the prevailing status of abiotic soil components. Linking soil ecosystem functioning to abiotic soil conditions will ultimately generate important information that can be used to facilitate the process of soil ecosystem restoration which is crucial to ensure sustainable crop production to feed nations around the globe.

CHAPTER 3: MATERIAL AND METHODS

3

3.1 Site description

3.1.1 Study localities and sampling sites

The two study localities (farms) are located in the eastern Free State (South Africa) near the towns of Vrede and Reitz, respectively. Each farm presented distinct differences in terms of agricultural management practices and soil physical characteristics (see Section 3.1.4). Both study localities are part of the GrainSA and Maize Trust Farmer Innovation Program (FIP) (https://www.grainsa.co.za/pages/grain-research/conservation-agriculture/farmer-innovation-programme). This program uses farmers practising conservation agriculture (CA) as pioneers through the inclusion of their farms in research projects to generate and spread knowledge about CA implementation in their respective areas. At each locality (Figure 3.1), five sampling sites were selected for investigations and included three CA farmlands, one conventional agriculture (Conv) farmland, and a natural veld (NV) area. The NV and Conv farmland served as pristine and degraded reference sites, respectively. Farmlands are referred to as treatments throughout this chapter.

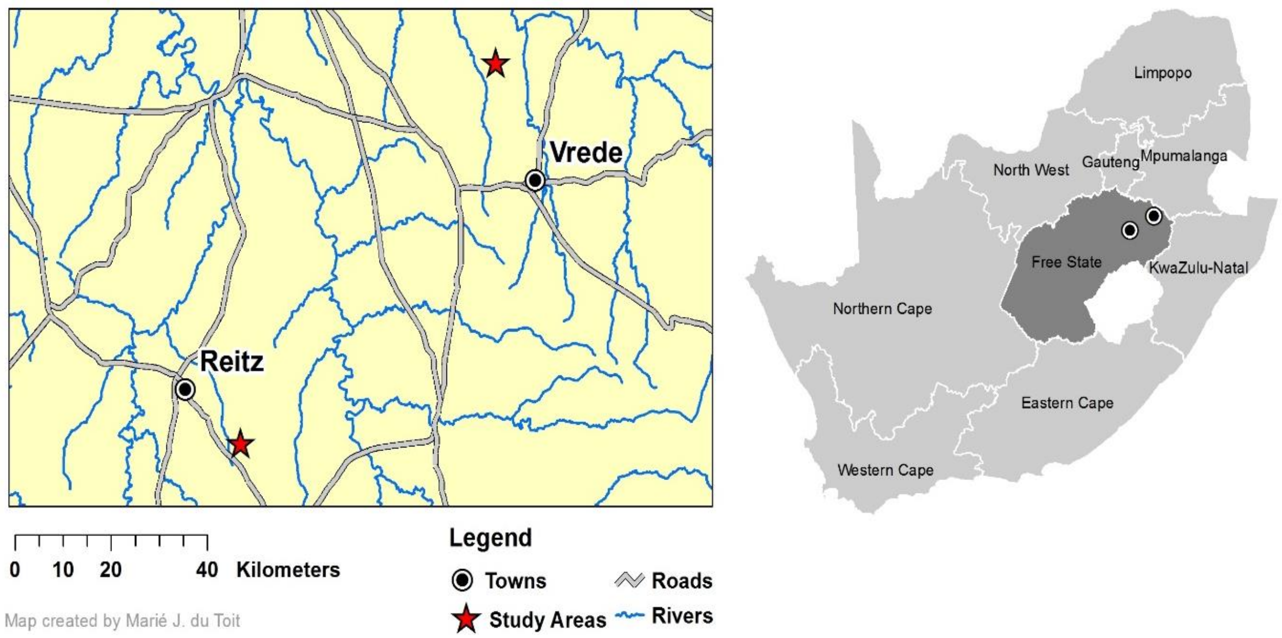


Figure 3.1: Location of the two study localities (Vrede and Reitz) in the Free State province of South Africa where this nematode-based study was conducted (Map created by Marié J du Toit, NWU).

The location information for the five sampling sites at each locality is presented in Table 3.1. Sampling sites were selected based on the implemented agricultural management practices (i.e., CA and Conv practices) and the availability of historical management information on the farmlands and NV areas (e.g., crop rotation patterns and fertilization, if applicable). The two localities were studied independently since substantial differences existed between the localities in terms of management practices and soil physical characteristics.

Table 3.1: Vrede and Reitz location information on the sites where soil samples were collected for biotic and abiotic analyses as part of a nematode study. The sampling sites at each locality included three conservation agriculture (CA) farmlands, one conventional agriculture (Conv) farmland, and a natural veld (NV) area.

Vrede (V)	**Site coordinates**	**Reitz (R)**	**Site coordinates**
First CA farmland (VCA1)	27°13'29.34"S 29°4'25.75"E	First CA farmland (RCA1)	27°52'36.55"S 28°32'42.43"E
Second CA farmland (VCA2)	27°13'46.06"S 29°4'24.04"E	Second CA farmland (RCA2)	27°53'26.07"S 28°32'51.84"E
Third CA farmland (VCA3)	27°13'17.99"S 29°4'31.90"E	Third CA farmland (RCA3)	27°53'6.23"S 28°32'28.67"E
Conv farmland (VConv)	27°13'33.52"S 29°4'7.85"E	Conv farmland (RConv)	27°54'30.48"S 28°32'4.19"E
Natural veld area (NVV)	27°13'24.08"S 29°4'33.58"E	Natural veld area (NVR)	27°53'58.20"S 28°31'56.16"E

3.1.2 Environmental conditions

3.1.2.1 Climatic conditions

It is important to consider the climatic conditions (e.g., temperature and precipitation trends) of geographical regions when conducting studies on agricultural production systems. The Free State province (a semi-arid region) is situated in a summer rainfall region (Adisa *et al.*, 2017) and although the province presents a wide precipitation range, it is mainly regarded as water-deficient (Hensley *et al.*, 2006). In a study on the effect of agro-climatic parameters (minimum and maximum temperature, potential evapotranspiration, and precipitation) on maize (*Zea mays*) production in South Africa, it was highlighted that maximum temperature was the main parameter that adversely affected maize yield in the Free State (Adisa *et al.*, 2017). Since the temperature of a region is a major climatic parameter (Moeletsi, 2017) it is important to note that the two study localities are subject to high maximum temperatures during the growing season. Tolerant crop varieties are therefore chosen accordingly for cultivation purposes (Adisa *et al.*, 2017). The two localities are ± 90 km apart with similar climatic conditions. The detailed climatic conditions of the Vrede and Reitz areas are shown in Table 3.2 [information obtained from WeatherSpark (2021 a,b)], while the monthly average precipitation over the previous 10 years (2010 - 2020) is provided in Figure 3.2. This figure highlights the similarity in terms of historic precipitation trends between the two study localities.

Table 3.2: A detailed description of the climatic conditions in Vrede and Reitz where a nematode study was conducted on the soil ecosystems of farmlands under different agricultural management practices.

Climatic characteristics	**Vrede**	**Reitz**
Summer conditions	Long, warm, and wet	Long, warm, and wet
Winter conditions	Short, cold, and dry	Short, cold, and dry
Annual temperature variation	-2 °C to 22 °C	-2 °C to 26 °C
Warm season	October to March	October to March
Cold season	May to July	May to August
Growing season*	Early September to early May (± 245 days)	Early September to early May (± 243 days)

*The longest continuous period where minimum temperatures remain above freezing (0 °C).

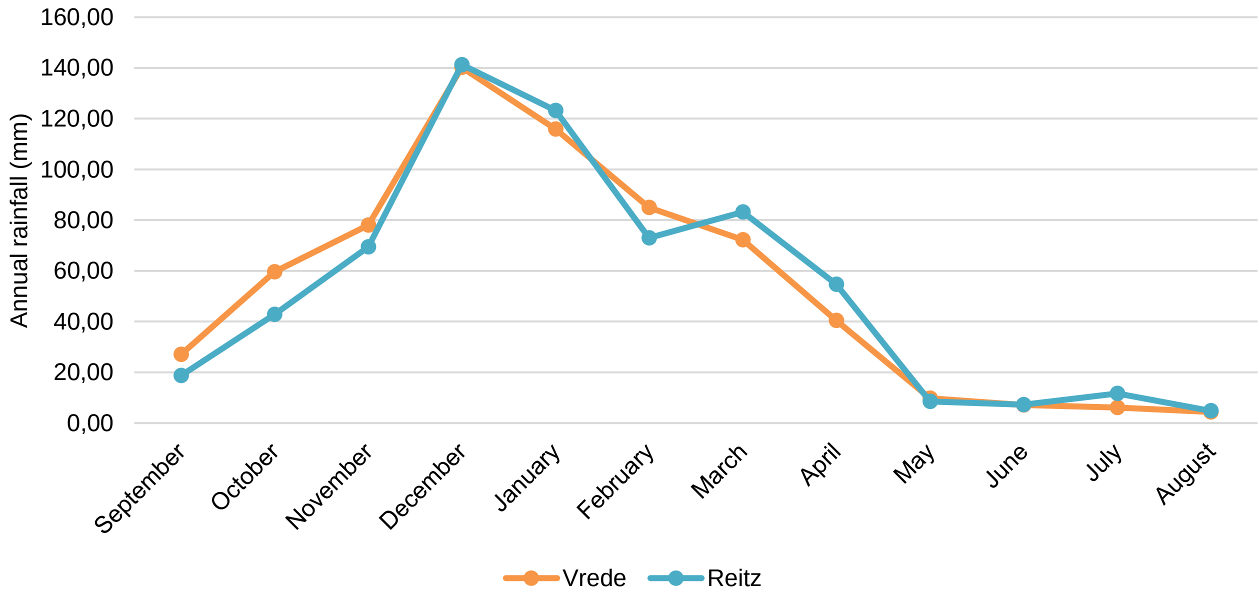


Figure 3.2: The average precipitation in Vrede and Reitz over the previous 10 years (2010-2020) was measured by a Dirk Friedhelm Mercker (DFM) mini weather station situated at each locality. Measurements were done by Free State Corporation limited / Vrystaat Koöperasie Beperk (VKB-Reitz).

Another factor that contributes to the climatic conditions of a region, is its altitude, latitude, and surrounding terrain (Conradie, 2012). The Vrede locality lies at ± 1600 m above sea level (WeatherSpark, 2021a) and Reitz at ± 1700 m above sea level (WeatherSpark, 2021b). Both localities form part of the moist Highveld Grassland climatic region, having a temperate climate resulting in warm summers and dry winters (Conradie, 2012).

3.1.2.2 Edaphic conditions

The plinthic soils of the north-eastern parts of the Free State, where this study was conducted, formed via intense weathering of Beaufort shale and mudstone-sedimentary rocks. There is furthermore a considerable contribution of sandy material, which originated from the Elliot, Molteno, and Clarens formations (Roux *et al.*, 2010).

3.1.3 Design of studied agricultural systems

The growing season in the eastern parts of the Free State typically spans from 140 to >160 days. Planting dates range from the beginning of October to mid-November (Moeletsi, 2017). This allows for the cultivation of medium to long growing maize varieties, planted at the end of October to mid-November, to mature in January and be physiologically ripe in March. However, farmers are advised not to plant their crops too late in the growing season as plants may be in danger of not fully maturing before the onset of the first frost (Moeletsi *et al.*, 2016).

3.1.3.1 Vrede

The Vrede sampling sites have a similar soil texture, namely clay, as was previously recorded by the FIP GrainSA project. The soil texture class and applicable inorganic fertilizer inputs of the farmlands in Vrede are presented in Table 3.3. It is important to consider soil texture and chemical inputs as it is known to influence soil biology (Naveed *et al.*, 2016).

Table 3.3: A summary of the soil texture class and inorganic fertilizer inputs of three conservation agriculture farmlands (VCA1-3), a conventional agriculture farmland (VConv), and a natural veld area (NVV) in Vrede before (first sampling interval) and during (second sampling interval) the summer growing season of 2019/20.

	Vrede (V)				
	VCA1	**VCA2**	**VCA3**	**VConv**	**NVV**
Soil texture class	Clay	Clay	Clay	Clay	Clay
First sampling interval crop	*WCC	WCC	WCC	None	None
First sampling interval fertilization	None	None	None	None	None
Second sampling interval crop	**SCC	Maize	SCC	Maize	None
Second sampling interval fertilization	None	N: 60 kg/ha P: 14 kg/ha K: 7 kg/ha	None	N: 110-115 kg/ha P: Not provided K: Not provided	None

*WCC = winter cover crops; **SCC = summer cover crops

The Vrede CA farmer aims to reduce the use of inorganic fertilizer and synthetic agrochemicals and has progressed in doing so over ± seven years. The Conv farmer, in turn, applied high amounts of inorganic fertilizer and synthetic agrochemicals. The planting of cash crops like maize and soybean has been alternated with summer cover crops (SCC) and the more recent cultivation of winter cover crops (WCC). It is important to note that the NV area, SCC, and WCC do not receive any inorganic fertilization.

The main CA practices implemented on the Vrede CA farmlands are no-tillage, cash crop rotation, and cover crop cultivation with livestock integration. Additionally, when fast-growing varieties of summer cash crops like soybean (*Glycine max*) are planted during the growing season, their short growth length provides sufficient time for the cultivation of a WCC. This WCC still benefits from the availability of adequate soil moisture and receives the required heat units for germination and crop growth. The crop rotation pattern and grazing periods for Vrede from the 2017/18 growing season to the 2019/20 growing season are presented in Table 3.4.

Table 3.4: Crop rotation [cash crop, summer cover crop (SCC), or winter cover crop (WCC)] and grazing practices of three conservation agriculture farmlands (VCA1-3), a conventional agriculture farmland (VConv), and a natural veld area (NVV) in Vrede from the 2017/18 growing season to the 2019/20 growing season. *Sampling interval.

Season	VCA1	VCA2	VCA3	VConv	NVV
2017/18 Summer crop	Maize	SCC	Maize	Maize	Natural veld
2017/18 Summer grazing	None	Cattle	None	None	Cattle
2018 Winter crop	None	None	None	None	Natural veld
2018 Winter grazing	Cattle	Cattle	Cattle	Cattle	None
2018/19 Summer crop	Soybean	Soybean	Soybean	Soybean	Natural veld
2018/19 Summer grazing	None	None	None	None	None
2019 Winter crop*	*WCC	WCC	WCC	None	Natural veld
2019 Winter grazing	Cattle	Cattle	Cattle	Sheep	Cattle
2019/20 Summer crop*	**SCC	Maize	SCC	Maize	Natural veld
2019/20 Summer grazing	Cattle	None	Cattle	None	Cattle

**WCC = winter cover crops; **SCC = summer cover crops

The three VCA farmlands have been under a cash crop and SCC rotation from 2012, while a WCC was planted for the first time in 2019. Cash crop residues are selectively grazed by cattle post-harvest as required and SCC are grazed toward the end of the growing season (February/March) before the seed formation stage. Whereas the WCC were grazed during September 2019 for utilization before the next growing season commenced. Selective grazing is implemented on all treatments in Vrede, where cattle can freely graze SCC, WCC, or cash crop residues until at least 30 % soil cover remains where possible.

Three different SCC mixes, each with a specific growth length/growth class were made available to the farmer by his local cover crop supplier, namely Barenbrug (https://www.barenbrug.co.za). The quantities of the different cover crops were expressed as kg/ha. An early planting SCC mix consisted of 10 kg babala (*Pennisetum americanum*), 4 kg oat (*Avena sativa*), 2 kg sorghum (*Sorghum bicolor*), 4 kg buckwheat (*Fagopyrum esculentum*), and 4 kg pea (*Pisum* sp.). A middle-class/medium growth length SCC mix of 4 kg sorghum or Sudan grass (*Sorghum × drummondii*), 2 kg babala, 10 kg cowpea (*Vigna unguiculata*), 4 kg dolichos (*Lablab purpureus*), 3 kg sunn hemp (*Crotalaria juncea*), 1 kg radish (*Raphanus sativus*), 4 kg oat, and 4 kg buckwheat. The late planting SCC, in turn, consisted of 12 kg maize, 5 kg grain sorghum (*Sorghum bicolor*), 5 kg wheat sorghum (*Triticum* sp.), 3 kg sunflower (*Helianthus annuus*), 3 kg oat, and 0.75 kg radish. The WCC cultivated on all three CA farmlands in 2019 included 20 kg oat, 8 kg rye (*Secale cereale*), 1 kg Japanese radish (*Raphanus sativus var. Longipinnatus*), 3 kg vetch (*Vicia* sp.), and 0.5 kg turnip (*Brassica rapa* subsp*. rapa*).

During the 2019/20 growing season, the farmer chose to cultivate the medium growth length SCC mix on his two CA farmlands VCA1 and VCA3. The medium growth SCC mix suited best with the climatic conditions of the growing season at the time, as a combination of the three growth lengths has been experimented with in previous years. The third CA farmland (VCA2) was under maize cultivation as it was in rotation with SCC from the previous year. Medium growing maize varieties are planted in October and allowed to grow for >140 days to maturity. The VConv farmland is under conventional tillage and continuous soybean-maize cash crop rotation since 2016. Before 2016, previous landowners planted either soybean or sunflower. Post-harvest cash crop residues of this farmland are grazed by sheep until all residues are utilized. There is no cultivation of SCC or WCC, nor a diverse crop rotation pattern on VConv. The NVV treatment is left undisturbed during most of the year, no cash or cover crops are cultivated, and no physical disturbance of the soil occurs with agricultural equipment. This treatment is grazed by cattle in conjunction with the CA farmlands, as these areas are located in proximity.

3.1.3.2 Reitz

The five sampling sites at Reitz presented large variation in clay content between the treatments and were consequently classified into differing soil texture classes, opposed to the uniform, soil texture found at the Vrede locality. As discussed in the Vrede Section, soil texture has substantial effects on soil ecosystems. Therefore, in this study, the specific soil texture class of each treatment was taken into account when interpreting soil ecosystem functioning results of the Reitz locality. The Reitz CA farmer also aims towards reducing synthetic agrochemical and inorganic fertilizer inputs, and through personal communication explained that a reduction of between 15 and 20 % in fertilizer inputs has already been achieved in comparison with Conv. The different soil texture classes and applicable inorganic fertilizer inputs of the treatments in Reitz are presented in Table 3.5

Table 3.5: A summary of the soil texture class and inorganic fertilizer inputs of three conservation agriculture farmlands (RCA1-3), a conventional agriculture farmland (RConv), and a natural veld area (NVR) in Reitz before (First sampling interval) and during (Second sampling interval) the growing season of 2019/20.

	Reitz (R)				
	RCA1	**RCA2**	**RCA3**	**RConv**	**NVR**
Soil texture class	Sandy loam/loamy sand	Sandy loam	Sandy clay loam	Sandy loam	Sandy loam
First sampling interval crop	None	None	None	None	None
First sampling interval fertilization	None	None	None	None	None
Second sampling interval crop	Maize	Maize	Maize	Maize	None
Second sampling interval fertilization	N: 80 kg/ha P: 20 kg/ha K: 20 kg/ha	N: 80 kg/ha P: 20 kg/ha K: 20 kg/ha	N: 80 kg/ha P: 20 kg/ha K: 20 kg/ha	N: 110 kg/ha P: 30 kg/ha K: 30 kg/ha	None

The Reitz CA farmlands are under a continuous annual cash crop rotation of sunflower, maize, and soybean, with the occasional cultivation of winter wheat (*Triticum aestivum*). Implemented cash crop rotation patterns and grazing periods for all treatments in Reitz from the 2017/18 growing season to the 2019/20 growing season are presented in Table 3.6. Cash crop rotation is a profitable practice for the Reitz CA farmer as the cultivation of a wide range of cash crops, including winter wheat, provides sufficient annual income and has added benefits to the soil ecosystem. These benefits include the disruption of pest and pathogen life cycles and a reduced need for inorganic fertilizer and synthetic agrochemicals.

The cash crop residues of the CA and Conv farmlands are selectively grazed to provide feed for either cattle or sheep during the dry season. The RConv farmland is mainly under maize mono-cropping, with soybean cultivation for the first time during the 2018/19 growing season. This farmland is under conventional tillage, where the soil is intensively tilled before planting and where tillage is additionally used for weed management. The Conv farmer also applies standard amounts of inorganic fertilizer during every growing season of maize cultivation. The NVR treatment is under ultra-high-density grazing (UHDG), a restorative grazing practice that allows the grazing of a small area by a large livestock herd for a short time (usually in February) whereafter a long recovery period (up to one year) is allowed for the grazed area.

Table 3.6: Summer and winter cash crop rotation and grazing practices of three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv), and one natural veld (NVR) area in Reitz from the 2017/18 growing season to the 2019/20 growing season. *Sampling interval. Acronyms used include UHDG: Ultra-high-density grazing and V Wheat: Volunteer wheat.

Season	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
2017/18 Summer crop	Soybean	Soybean	Soybean	Maize	Natural veld
2017/18 Summer grazing	None	None	None	None	UHDG
2018 Winter crop	Wheat	Wheat	Wheat	None	Natural veld
2018 Winter grazing	Cattle	Cattle	Cattle	Cattle/sheep	Rest and grow
2018/19 Summer crop	Sunflower	Sunflower	Sunflower	Soybean	Natural veld
2018/19 Summer grazing	None	None	None	None	UHDG
2019 Winter crop*	*V Wheat	V wheat	V wheat	None	Natural veld
2019 Winter grazing	Cattle	Cattle	Cattle	Cattle/sheep	Rest and grow
2019/20 Summer crop*	Maize	Maize	Maize	Maize	Natural veld
2019/20 Summer grazing	None	None	None	None	UHDG

* Volunteer wheat

3.2 Sampling methodology

3.2.1 Sampling intervals

Soil sampling took place during two sampling intervals at both study localities. The first sampling interval occurred at the beginning of September 2019 before the summer growing season i.e., before planting. In Vrede, residues of the grazed WCC were present on the VCA farmlands. On the VConv farmland, soybean residues were entirely utilized by sheep during the winter. The NVV area was grazed by cattle simultaneous with the grazing of the WCC on the VCA farmlands during the winter and was, therefore, left undisturbed at the time of sampling. At Reitz, sunflower residues from the 2018/19 summer growing season and volunteer wheat residues from the 2018 winter were present on the RCA farmlands during the first sampling interval. Crop residues were already selectively grazed by cattle during the winter months that passed. The RConv farmland contained soybean residues, which were also selectively grazed by sheep during the winter. The NVR area was undisturbed at the first sampling interval as it was in its recovery period from UHDG, which occurs every year during February/March. The second sampling interval occurred in mid-February 2020 during the summer growing season. In Vrede, two of the three VCA farmlands (VCA1 and VCA3) had a medium growing length SCC (Table 3.4) while the other VCA farmland (VCA2) had maize in the R2 reproductive stage. The VConv farmland had maize in the vegetative tasselling (VT) stage and the NVV area was undisturbed as sampling occurred before its simultaneous grazing with the adjacent SCC farmlands in March. In Reitz, during the second sampling interval, all three RCA farmlands had maize in the flowering and ear forming R2 reproductive stages. The RConv farmland had maize in the vegetative growth V12 stage and the NVR area was not yet grazed and therefore undisturbed to be under UHDG at the end of February/beginning March.

3.2.2 Soil sampling for biotic and abiotic analyses

Six replicate soil samples were taken at each sampling site (i.e., CA farmland or NV area). The positioning of the replicates (Figure 3.3 a) was set using the unaligned grid sampling approach (Peters *et al.*, 2007; Wollenhaupt & Wolkowski, 1994). Each of the six sample replicates further consisted of 10 randomized sub-samples, with each taken in a radius of 5 m around the centre point, which also served as the first sub-sampling point (Figure 3.3 b).

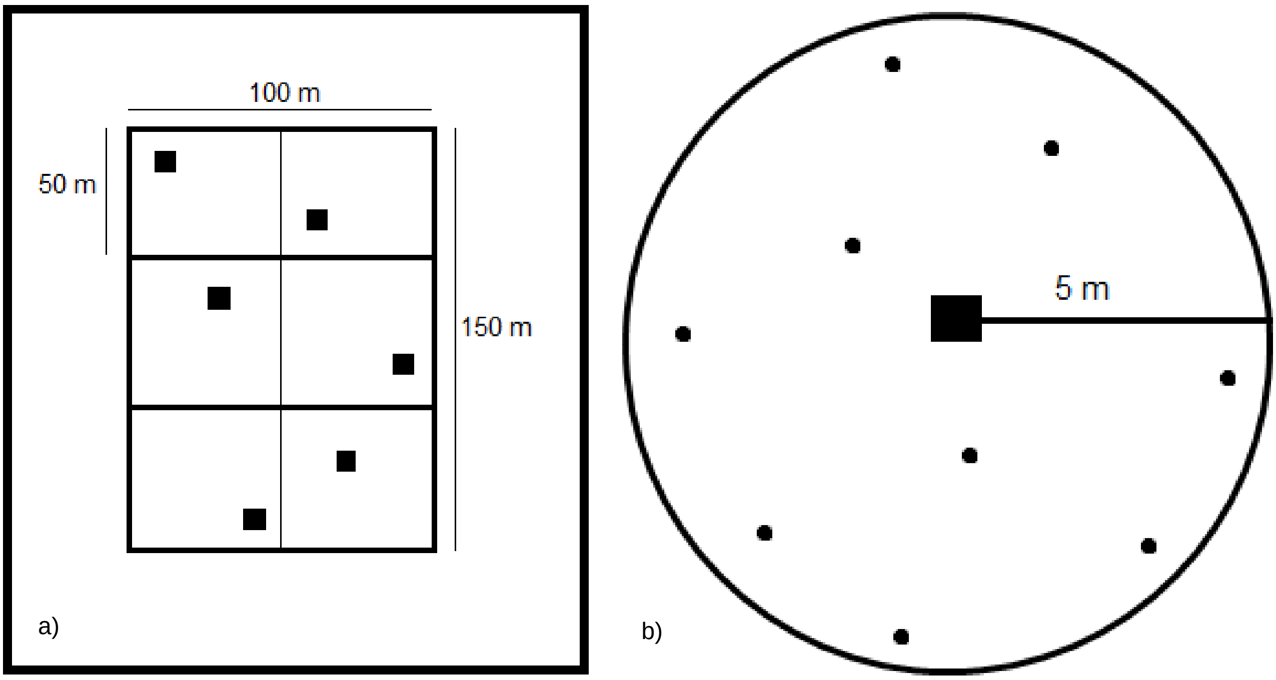


Figure 3.3: a) An example of unaligned sampling points plotted on a 100 x 150 m grid that was placed within the borders of conservation and conventional agriculture farmlands, as well as natural veld areas, at the two study localities (Reitz and Vrede). b) An illustration of the 10 randomized sub-sampling points in a 5 m radius around each pre-determined sampling point. The centre point (square) served as the first sub-sampling point (Illustrations: Ané Loggenberg, NWU).

Unaligned grid sampling requires that sampling points are not located on the same horizontal and vertical lines (Peters *et al.*, 2007). As a result, sampling points are evenly spread over the sampling area. This approach was used as it ensures that representative samples are taken from each site and that excessive variation between samples is minimized (Mulla & Khosla, 2016; Wollenhaupt & Wolkowski, 1994). First, a 100 m x 150 m grid consisting of six 50 m x 50 m blocks were drawn over each sampling site (*viz*. CA, Conv, and NV). The grid and GPS coordinates of each sample replicate were determined using Google Earth Pro (https://earth.google.com). For each sub-sample, the top 20 cm of soil (Marais *et al.*, 2017) were taken with the use of a soil auger (Figure 3.4 a). The content of the auger was emptied into a bucket (Figure 3.4b). Upon completion, the content of the bucket containing all 10 sub-samples was homogenized using a small spade. One litre (L) of the homogenized soil was added to a sampling bag labelled "Biotic" and another 1 L to an "Abiotic" labelled bag. The labels further contained the sampling site name (i.e., VCA1 or RCA1) and replicate indication (i.e., a-f). The abiotic and biotic samples of each sampling site were added to separate polystyrene cooler boxes and kept out of direct sunlight (Marais *et al.*, 2017). Hereafter samples were transported to the North-West University's (NWU) Nematology Diagnostic Laboratory to be stored at 6-10 °C (Marais *et al.*, 2017) until further processing.

Figure 3.4: a) Soil sampling with the use of soil augers at a sampling site in Vrede (Photo: Dr. Gerhard du Preez, NWU). b) A handful of homogenized soil from ten sub-samples taken at a sampling site in Vrede (Photo: Kelebogile Motlhamme, NWU).

3.2.3 Sample preparation for biotic and abiotic analyses

The abiotic soil samples were air-dried (Figure 3.5 a) for 48-96 h at 30 °C within one week after sampling. Next, the soil samples were lightly crushed and sieved through respective mesh sizes (4.75 mm and 2 mm) (Figure 3.5 b) as required by each analysis protocol (Schindelbeck, 2016).

Figure 3.5: a) Abiotic soil samples placed in an oven to be air-dried for 48-96 hours at 30^{0}C. b) Soil sieves with their respective mesh sizes used to sieve the soil to the required fractions for analysis. (Photos: Ané Loggenberg, NWU).

Soil respiration (SR) required soil sieved through a 4.75 mm mesh, while active carbon (AC) and % organic matter (% OM) required soil sieved through a 2 mm mesh. The latter fraction was also required for determining the soil physical parameters, namely bulk density, and soil texture. The soil chemical parameters electrical conductivity (EC), pH, soil available phosphorus (P), as well as soil micro- and macronutrients, were also measured using 2mm sieved soil. The necessary amount (g) of soil from each sieved fraction was removed and stored in air-tight containers at room temperature until further use.

3.3 Soil ecosystem analysis

3.3.1 Analysing the state of soil ecosystems using nematodes as bioindicators

Nematode-based indices (NBIs) are used to measure the status of the soil food web and are an accurate indicator of soil ecosystem health and functioning (Reichel *et al.*, 2017). In this study, these indices were used to increase the resolution of soil ecosystem functioning measurements in terms of the impact that agricultural management practices have on soil ecosystem functioning in farmlands.

3.3.1.1 Soil nematode extraction

Before nematode extraction, the soil in each biotic sample bag was homogenized after which 200 g was removed and placed in a one L plastic container with 500 ml water. Samples were left to soak for 24 h at room temperature to help break apart soil aggregates during the extraction process. Soil nematodes were extracted using an adapted version of the decanting and sieving and sugar centrifugal flotation method (Marais *et al.* (2017). The decanting and sieving method separates nematodes from soil particles based on their specific gravity (1.08 g cm^{-3}), which is less than that of soil particles. This causes nematodes to stay in suspension and be separated as they float above most of the soil particles of the sample. Following is the centrifugal sugar flotation method, which also causes nematodes to float and other particles to sink to the bottom of a centrifuge tube with the addition of a sugar solution that has higher specific gravity than nematodes (1.15 g cm^{-3}).

Each weighed and soaked soil sample was first rinsed from its container into a 2 L beaker, which was filled to a volume of 1 L with a strong flow of tap water. This caused the disruption and thorough mixing of the soil with water. The beaker was left to stand for 15 s, whereafter the water, suspended plant material, and soil particles were poured over a 2 mm kitchen sieve into a 5 L bucket. Care was taken not to pour the already settled particles over the kitchen sieve. This process was repeated three times, each time after a waiting period of 15 s. The content of the 5 L bucket was poured over a 25 µm sieve and rinsed into a 500 ml glass beaker not exceeding the 100 ml mark. The content of the glass beaker was evenly distributed into four 50 ml centrifuge tubes. One teaspoon (5 g) of kaolin was added to each centrifuge tube, topped up with tap water, and mixed with a spatula. The four tubes containing kaolin were centrifuged at 3500 rpm for 5 min. Kaolin aids the formation of a pellet at the bottom of the tube containing larger unwanted particles, while the lighter organic matter remains in suspension. After centrifugation, the content of the tubes was discarded, this included organic material with a specific gravity < 1 g cm^{-3} (Marais *et al.*, 2017).

Next, a sugar solution with a specific gravity of 1.15 g cm^{-3} was prepared by dissolving 1.6 kg of sugar in 2 L water. The centrifuge tubes were filled with the sugar solution and well mixed with a spatula to dislodge and disaggregate the pellet (the spatula was rinsed between individual samples to prevent contamination). Centrifuge tubes were filled with the sugar solution to suspend nematodes during a second centrifugation, which took place for 1 min at 3500 rpm. Nematodes are suspended due to the difference between the specific gravity of terrestrial nematodes (1.08 g cm^{-3}) and the sugar solution (Marais *et al.*, 2017). The content of the tubes was rinsed through a 25 µm sieve to remove the sugar solution from the nematode sample. Care was taken to quickly remove the sugar solution from samples as prolonged exposure of nematodes to the sugar solution may cause structural damage to the nematode body due to the increased osmotic pressure. The extracted sample was rinsed with ± 20 ml water into a labelled sample bottle and stored at 6 °C.

3.3.1.2 Nematode fixation with 4 % formaldehyde

To prevent structural degradation of the nematode body, nematodes present in the samples were fixed using formaldehyde (Marais *et al.*, 2017). An amount of 20 ml warm (80 °C) and 20 ml of a cold (4 °C) 6 % formaldehyde solution was added to each 20 ml nematode sample. The dilution resulted in a final formaldehyde concentration of 4 % (i.e., 10 % formalin). Samples were poured into 50 ml falcon tubes. The falcon tubes containing the samples were placed in an upright position for 48 h to allow nematodes to settle to the bottom. The majority of the solution was carefully abstracted from at the meniscus level of the sample using a plastic pipette. Care was taken not to disturb nematodes settled at the bottom. Only 8 ml solution containing the nematodes remained in the falcon tubes, while the abstracted was checked under a stereo microscope (Nikon C-DS) to ensure that no nematodes were accidentally removed. Next, the total number of nematodes in the 8 ml sample was counted after which the sample was transported to a labelled 15 ml falcon tube (Figure 3.6 a). These falcon tubes were left in an upright position for 48 h before another 6 ml of the solution was abstracted using the same process as before. The abstracted solution was also checked for nematodes. The nematodes settled at the bottom of the remaining 2 ml solution were used for identification and counting.

3.3.1.3 Nematode mounting on macro slides

Nematodes fixed with formaldehyde were mounted on permanent glass macro slides (76 x 50 mm) for easy and accurate morphological identification. A paraffin wax square (35 mm) was pressed onto the macro slide using a heated metal tube. Following, a nematode sample was vigorously shaken and 80 µl removed using a 200 µl pipette. The aliquot was pipetted to the centre of the wax square, which was then covered with a glass coverslip (45 x 45 mm). The macro slide was placed on a hotplate with low heat to gradually melt the wax below the coverslip. This resulted in the nematode sample being sealed in the middle of the glass slide. All sides of the coverslip were secured with three layers of transparent nail polish to prevent leakage (Figure 3.6 b). The permanent macro slides were labelled and stored in a horizontal position.

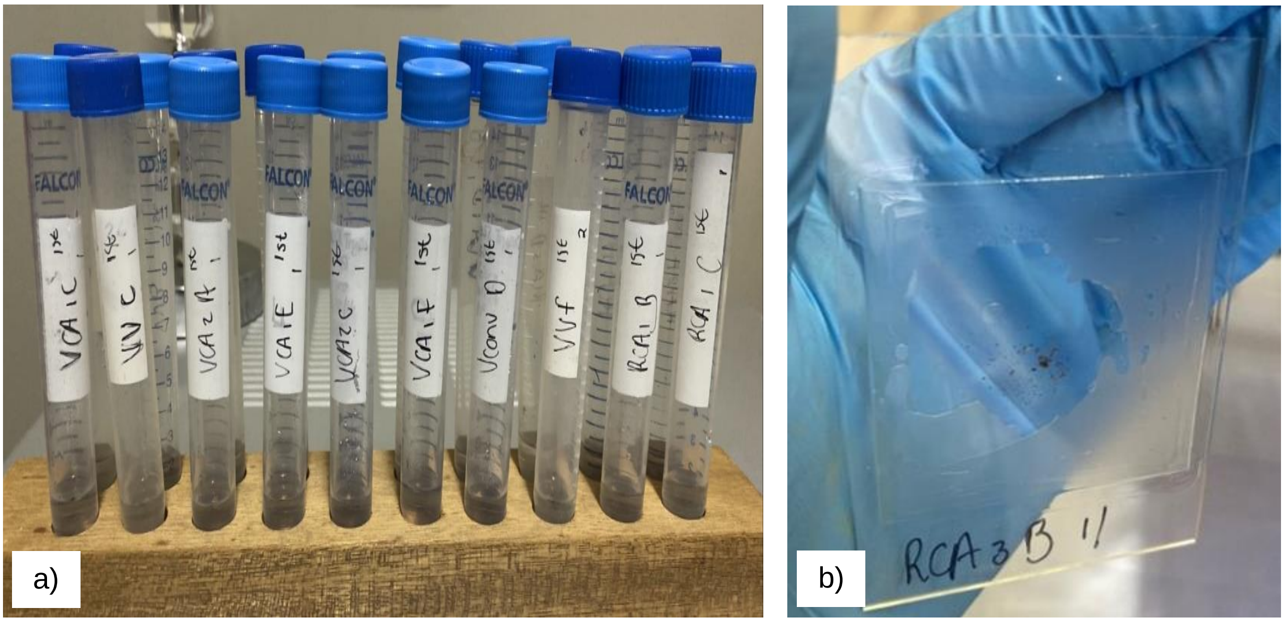


Figure 3.6 a) The 15 ml falcon tubes with 2 ml of 4 % formaldehyde solution containing the fixed nematodes after they were extracted from soil samples. b) A coverslip sealing the 80 µl sample containing nematodes fixed in 4 % formaldehyde (Photos: Ané Loggenberg, NWU).

The first 100 nematodes from each sample were counted and identified to genus level (sometimes family level if a nematode individual was partly degraded) using a Novel N-400M light microscope manufactured by Nanjing Jiangnan Novel in China. Identification was done under 40-1000x magnification. Guides used for nematode identification included Heyns (1971), Andrássy (2005), Andrássy (2007), and Andrássy (2009) with their accompanying keys.

3.3.2 Analysing the state of soil ecosystems using bioindicators

Biological indicators of soil ecosystem functioning that were used in this study include SR, AC, and % OM (Moebius-Clune *et al.*, 2016; Van Es & Karlen, 2019). Nematode-based indices (NBIs) furthermore were also used to indicate soil ecosystem restoration of farmlands and are discussed in Section 3.3.2. The metabolic activity of soil organisms is represented by SR (Moebius-Clune *et al.*, 2016). It further reflects the rate of OM mineralization and nutrient availability in the soil ecosystem (Fierer, 2017; Lazcano *et al.*, 2021). The amount of carbon (C) that serves as a readily available food source for the soil microbial community is represented by measurements of AC. The amount and size of AC pools in the soil are an accurate indicator of short-term changes in the soil ecosystem (Nakamoto *et al.*, 2012; Weil *et al.*, 2003), while %OM affects greenhouse gas emission, nutrient cycling, water filtration, and soil biomass production in the soil ecosystem (Bünemann *et al.*, 2018; Seaton *et al.*, 2020).

3.3.2.1 Soil respiration

The protocol for measuring SR was obtained from Dr. Rick Haney, who supplied a Minicube for SR measurements. Two replicates of each sample were measured and samples with differences in CO2-C concentration greater than 5 % from the average mean of the measured samples were re-measured (Schindelbeck, 2016). First, a single 0.2 V Whatmann filter paper was cut to fit the bottom of a small 50 ml plastic beaker. Beforehand, three evenly spaced holes (6 mm diameter) were drilled into the bottom of the beaker. A single filter paper was added and thereafter 40 g of the 4.75 mm sieved soil. Next, 16 ml deionized water was added to a 250 ml glass jar. The 50 ml beaker containing the 40 g soil was placed inside the 250 ml jar. This resulted in the rewetting of the soil from the bottom.

The glass jar was sealed with an air-tight lid fitted with two quick connectors that are closed with plugs (Figure 3.7a). These connector plugs were sealed with petroleum jelly, while the lid of the jar was sealed with parafilm. The 250 ml jars were incubated for 24 h at 25 °C. After incubation, the SR (ppm) was measured. This was done by connecting the quick connectors of each jar to the Minicube as shown in Figure 3.7 b. The Minicube was connected to a computer and the CO_2 concentration that resulted from respiration by organisms in the wetted soil was measured using GasLab 2.1 USDA CO_2 Sensor software.

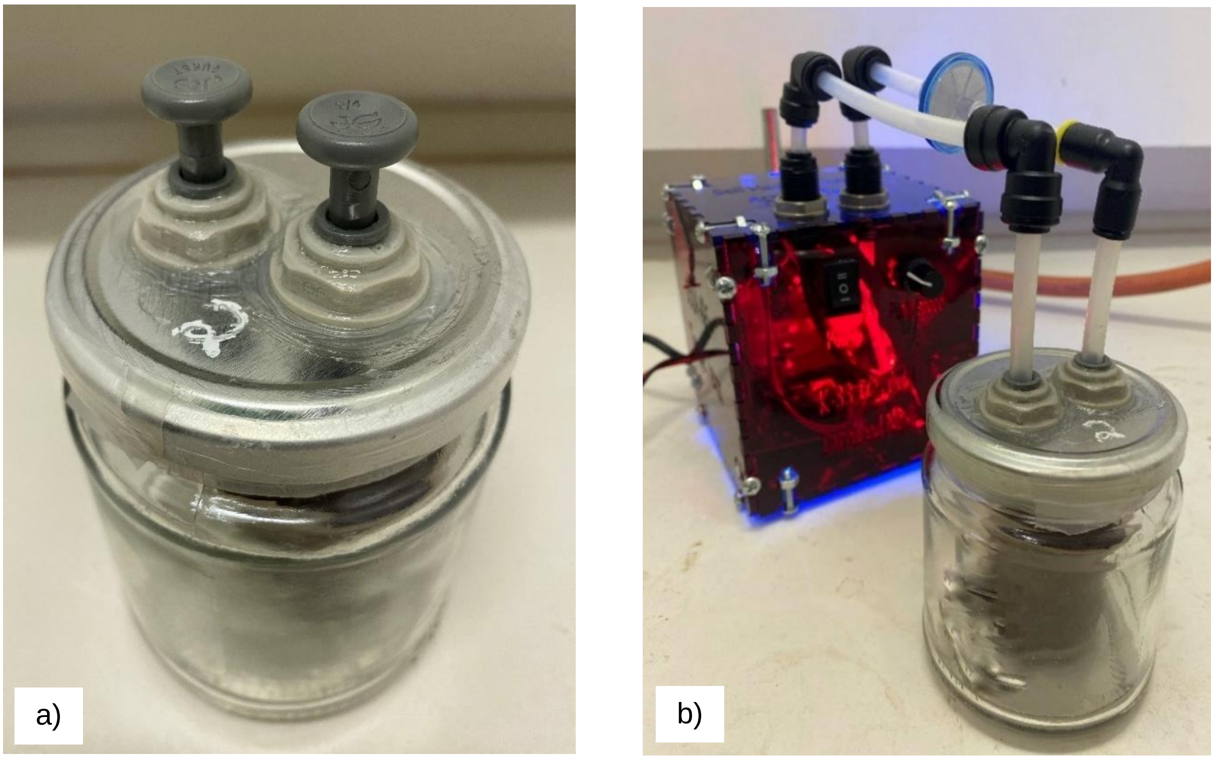


Figure 3.7 a) The quick connectors with plugs mounted on the lids of 250 ml jars that are used in the incubation of soil samples to measure soil respiration. b) A 250 ml jar connected to a Minicube that measures soil respiration (Photos: Ané Loggenberg, NWU).

3.3.2.2 Active carbon

The standard operating procedures (SOPs) of Cornell University (Schindelbeck, 2016) was used for AC measurements. A 0.2M $KMnO_4$ stock solution was prepared in a 2 L glass beaker covered with an opaque paper bag to prevent light exposure. This was done by dissolving 11.09 g $CaCl_2$ in 750 ml deionized water with the help of a magnetic stirrer. Next, 31.61 g $KMnO_4$ was added to the solution along with 200 ml deionized water after which the solution was stirred continuously until all the reagents dissolved (±1 h). A calibrated pH meter (Mettler Toledo FiveEasy™ Benchtop pH Meter) was used to measure the pH of the solution, which was adjusted with the addition of 0.1 N HCl (to decrease the pH) or KOH (to increase the pH) until the pH stabilized at a reading of 7.2. Thereafter, the stock solution was poured into a volumetric flask and made up to a volume of 1000 ml with deionized water. The stock solution was stored in a labelled opaque container to ensure stability for a maximum of three months.

Before measurement, the standard curve for the stock solution was determined. A spectrophotometer (Spectroquant® Pharo 300) was zeroed on 550 nm using deionized water. Three labelled falcon tubes were filled with 45 ml of deionized water. Deionized water was added to each falcon tube in sequence, tube 1; 3.75 ml; tube 2, 2.50 ml; and tube 3, 0.0 ml. Thereafter, 1.25 ml, 2.50 ml, and 5.00 ml of the 0.2 M $KMnO_4$ stock solution were added to each tube respectively. The final concentration of $KMnO_4$ in each 50 ml falcon tube was 0.005M, 0.01M, and 0.02M respectively. Each tube was capped and shaken for 10 s. Thereafter, 49.5 ml of deionized water was dispensed into nine additional clean 50 ml falcon tubes serving as triplicates for each standard concentration. From each standard, 0.5 ml was added to its set of triplicates after which the tubes were capped and shaken for 10 s. The absorbance of the triplicate samples was measured and recorded for each standard. These values were used to construct a linear regression model, which served as a standard curve for the $KMnO_4$ stock solution (Schindelbeck, 2016).

Two replicates of each soil sample were measured when the AC content of the soil samples was determined. Two replicates of each sample were measured and samples with differences in AC concentration greater than 5 % from the average mean of the measured samples were re-measured. A maximum of 10 samples was analysed per batch. Each replicate was assigned two falcon tubes containing 18 ml and 49.5 ml deionized water, respectively. The falcon tube containing 18 ml deionized water served as the reaction tube and the falcon tube containing 49.5 ml deionized water was used to measure the sample's final absorbance. The process started by adding 2.5 g of soil (weighed into small weigh boats) to the 18 ml reaction tube. Next, 2 ml of 0.2M $KMnO_4$ was added to the tubes to start the redox reaction. Care was taken to always start with the same sample and move in sequence toward the last sample. Each tube was capped tightly, briefly shaken, and placed on a shaker for 2 min at 120 rpm. Following, the samples were removed and sloshed to re-suspend the soil adhering to the sides of the tube. Next, the samples were uncapped on the benchtop and the stopwatch was left running for another 8 min resulting in a total reaction time of 10 min. After 10 min passed, the reaction was stopped by abstracting 0.5 ml from the reaction tube in the same sequence that the $KMnO_4$ was added. The 0.5 ml aliquot was transferred to the tube containing 49.5 ml deionized water as shown in Figure 3.8. Care was taken to rinse the pipette tip between transfers of different samples.

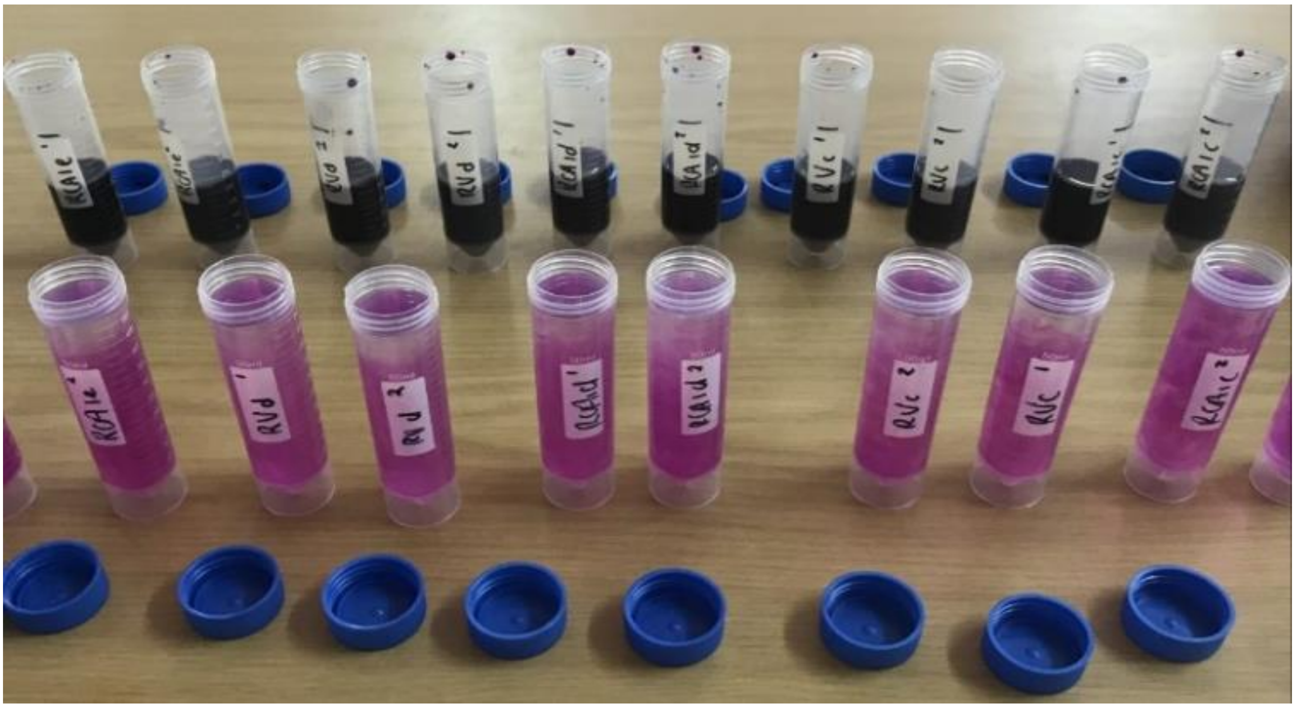


Figure 3.8 Falcon tubes containing 49.5 ml deionized water and a 0.5 ml aliquot (front row) added from the reaction tubes containing 18 ml deionized water, 2.5 g soil, and 2 ml 0.2M $KMnO_4$ (back row) as part of the protocol to measure the active carbon content of soils (Photo: Ané Loggenberg, NWU).

After the transfer was complete, the tubes with the diluted samples were capped and lightly shaken for 10 s. Each sample was lightly shaken before measurement. The cuvette was rinsed with a fraction of the sample, then filled, inserted, and measured. After measurement, the absorbance values for both replicates were recorded and the percentage difference was calculated.

For calculating the AC content of the samples, the concentration of AC is the sum of the slope (b) and y-intercept (a) of the linear regression equation. Concentration (y) is the dependent variable and absorbance (x) the independent variable: Concentration = a + b * (absorbance). The following formula was used to calculate the amount of AC in the sample:

Active C (mg/kg) = [0.02 mol/L - (a + b * absorbance)] * (9000 mg C/mol) * (0.02 L solution/0.0025 kg soil).

- 0.02 mol/L is the initial $KMnO_4$ concentration
- 9000 mg is the amount of carbon (0.75 mol) oxidized by 1 mol of MnO_4 (Mn^{+7} to Mn^{2+})

- 0.02 L is the volume of the $KMnO_4$ solution
- 0.0025 kg is the amount of soil used

3.3.2.3 Organic matter content

The protocol for measuring % OM was adapted from Van Reeuwijk (1986) and Matthiessen *et al.* (2005) and requires the use of a muffle furnace set to 450 °C. Clean porcelain crucibles were placed in the heated muffle furnace for 8 h. Thereafter they were removed and added to a desiccator to cool to room temperature for the crucible mass to be recorded. Each weighed and labelled crucible was filled with 10 g of 2 mm sieved, air-dried soil from the corresponding sample, and the total mass was recorded. Crucibles containing the air-dried soil were placed in the muffle furnace of which the temperature was increased in intervals of 50 °C (to avoid the thermal shock of the samples) until it reached 450 °C. The crucibles were removed from the furnace after 24 h and placed in a desiccator to cool after which the mass of each crucible was again recorded.

The calculation of % OM was done as follows: B-C/B-A*100

A: Crucible mass

B: Crucible + dry soil mass

C: Crucible + combusted soil sample

3.3.3 Analysis of physical and chemical indicators of soil health

The analysis protocol for soil texture, pH, and EC was obtained from the SOPs of the Cornell CASH framework written by Schindelbeck (2016). Inorganic nitrogen (N), essential plant macronutrients including calcium (Ca), magnesium (Mg), potassium (K), P, sulphur (S), and sodium (Na), micronutrients including aluminium (Al), boron (B), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) were analysed by Intertek Agricultural Laboratories (https://www.intertek.com).

3.3.3.1 Soil texture

The soil texture analysis required 2 mm sieved and air-dried soil of which 14 g was added to 24 ml of a 3 % sodium hexametaphosphate solution in a labelled 50 ml falcon tube. The exact mass (to four decimals) of the soil sample was recorded using an analytical balance. Next, the falcon tube was lightly shaken to suspend all the soil particles after which the falcon tubes were placed on a shaker for 2 h at 150 rpm. Sodium hexametaphosphate is a dispersing agent that helps in separating soil particles into their respective soil fractions. Following this, two porcelain crucibles were assigned to each sample. All crucibles were washed with a mild soap mixture, dried at 105 °C for 1 h, and left to cool to room temperature in a desiccator. The mass of each crucible was determined on an analytical balance and recorded. One labelled crucible was assigned to the sand fraction and the other to the silt fraction. After 2 h of shaking, the falcon tubes were lightly sloshed to separate any soil particles from the sides of the falcon tubes. The content of the tube, as well as any particles adhering to the cap, were rinsed through a sieve assembly, which consisted of a 53 µm sieve placed on top of a 20 cm diameter funnel draining into a 1 L beaker (Figure 3.9) While rinsing, circular movements of the fingers (while wearing gloves) were used to gently force all silt and clay particles through the sieve leaving only the sand fraction behind. Rinsing the silt and clay particles must be done with no more than 900 ml of water.

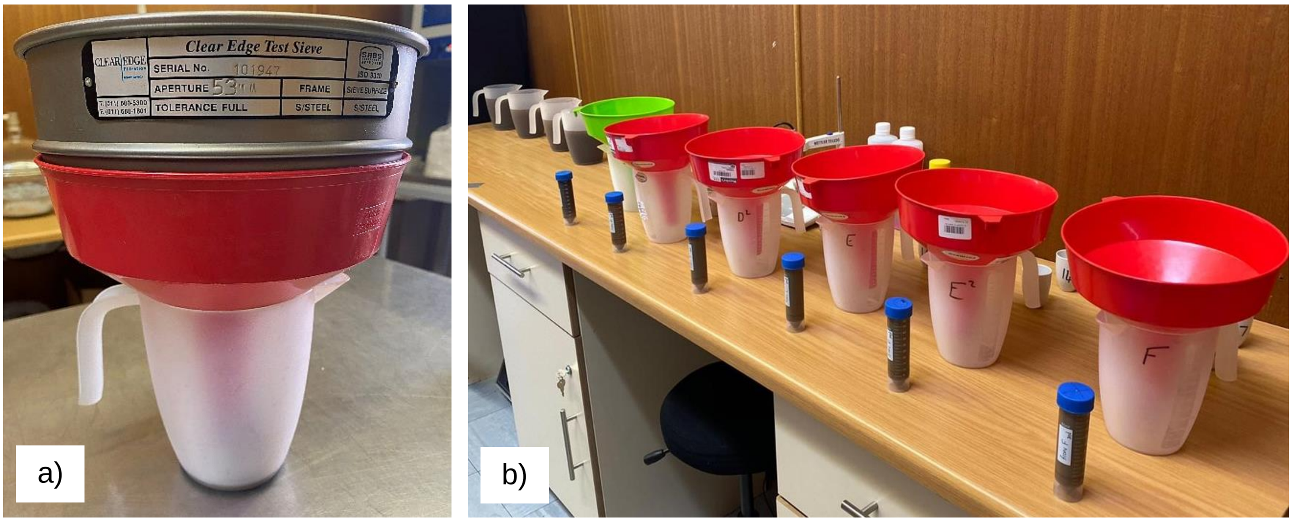


Figure 3.9: a) A sieve assembly consisting of a 53 µm sieve placed on top of a funnel that is inserted into a 1 L beaker. This assembly was used to separate the sand fraction from the silt and clay fractions during soil texture analysis. b) Soil texture protocol preparation including soil samples in falcon tubes containing 3 % hexametaphosphate (front row) and funnels inserted into designated 1 L beakers as part of a sieve assembly (back row) (Photos: Ané Loggenberg, NWU).

Sand particles collected on the sieve were carefully rinsed into the sand crucibles. Next, the content of the 1 L beaker was decanted into a temporary beaker and back into the original beaker. This caused the re-suspension of the silt and clay practices. The beaker was thereafter left undisturbed for 2 h to allow the settling of silt particles. Careful decanting of the suspended clay fraction into a waste bin was done, while care was taken not to disturb the silt fraction that accumulated at the bottom. Following, the silt fraction was rinsed into the silt crucible. The sand and silt crucibles were dried at 105 ˚C for 24 h after which they were placed in a desiccator to cool to room temperature. After cooling, the crucibles were weighed using an analytical balance. The percentages of sand and silt were calculated from the respective masses of the dried fractions compared to the initial mass of the samples. The remaining percentage thus represented the clay fraction. Soil texture was expressed as the percentage of sand, silt, and clay.

3.3.3.2 pH and EC

For each sample, sieved soil (10 ml) was added, together with 25 ml deionized water, to a 50 ml falcon tube. The tubes were placed on a shaker for 1 h after which each tube was sloshed to loosen the soil particles from the sides. All tubes were left to settle for 15 min after which the pH, EC, and temperature of the aliquot were measured using the Hanna HI 9811-5 portable pH meter.

3.3.3.3 Soil nutrient analysis

The protocols for all soil nutrient extractions were provided through personal communication with Intertek Agricultural Laboratories, Johannesburg, South Africa. The Mehlich III extraction was used to determine the Al, B, Ca, Co, Cu, K, Mg, Mn, Na, P, and Zn content of each soil sample (Mehlich, 1984). For this extraction, 2.5 ml of air-dried and 2 mm sieved soil was added to a 100 ml extraction bottle. Next, 1 ml activated C was added to ensure a colourless filtrate. Hereafter, 25 ml Mehlich III extraction solution was added, and the bottle was placed on a shaker at 200 rpm for 5 min. The sample was filtered through a Whatmann V2 filter paper and the filtrate was analysed. Quantification was done on an ICP – OES analyser.

Soil inorganic N in the form of NH_4 and NO_3 was extracted by using the equilibrium method (Mulvaney, 1996). This method required that 10 g of the 2 mm sieved air-dried soil was added to a 250 cm^3 extraction bottle. Hereafter, 100 cm^3 1.0 mol dm^3 KCl was added, a stopper was inserted, and the bottle was placed on a shaker at a moderate rpm for 30 min. The sample was filtered to obtain a clear extract. If the extract couldn't be analysed within 24 h it had to be refrigerated until measurement. The NH_4 and NO_3 content of the sample was determined on an Auto Analyzer Flow system.

3.4 Statistical analysis

Nematode community data were used to calculate NBIs with the NINJA: Nematode INdicator Joint Analysis Online Tool (Sieriebriennikov *et al.*, 2014). Calculated NBIs included the Basal index (BI), Channel index (CI), Enrichment index (EI), Structure index (SI), and Maturity Index 2-5 (MI 2-5), along with nematode metabolic footprints (MNF) consisting of the Bacterivore, Enrichment, Fungivore, Structure, Herbivore, Omnivore, and Predator footprints. Radial plots illustrating the NMFs were created using Microsoft Excel 365. Data for the plotting of radial plots were ln transformed (Lazcano *et al.*, 2021).

The NBIs calculated for each sampling interval, locality, and study site were also subjected to a Factorial Analyses of Variance (ANOVA) using TIBCO Statistica™ Version 14.0.0.15 Software Package (https://www.statistica.com.) This analysis was used to determine if the treatments, intervals, and interaction (Treatments*Interval) had a significant ($p<0.05$) effect on the NBIs. The assumptions of normality and homogeneity of variance were tested using normal probability plots and the Bartlett's test, respectively. If required, the data was Log_{10} (x+1) transformed. Significant differences between treatments and intervals were investigated using the Tukey HSD post-hoc test at $P = 0.05$.

Redundancy analyses (RDAs) were used to investigate the correlation between the measured abiotic (explanatory variables) and biotic (response variables) parameters, and the treatments (CA and Conv farmlands and NV areas), during both intervals at Vrede and Reitz. Explanatory variables for each RDA were selected using the Forward Selection method to include only those that best explain the observed variation in the response variables. Also, the predictive effects of the explanatory variables were calculated using a Monte-Carlo permutation test (Šmilauer & Lepš, 2014). The RDAs were performed and illustrated on biplots using Canoco 5 Software Package. Linear regression models were used to further investigate meaningful and significant correlations from the RDAs. These analyses were performed using Graphpad Prism 8 Software Package. Significance for all univariate analyses was regarded at $p<0.05$.

CHAPTER 4: VREDE RESULTS AND DISCUSSION

4

4.1 Introduction

The state and functioning of soil ecosystems are subject to change as a result of the implementation of agricultural management practices (Yang *et al.*, 2020). Conservation agriculture (CA) is known to aid in the restoration of soil ecosystem functioning in farmlands utilized for crop production (Page *et al.*, 2020; Swanepoel *et al.*, 2017). The three Vrede CA farmlands investigated in this study are under cover crop cultivation in annual rotation with a summer cash crop, e.g., maize (*Zea mays)* or soybean (*Glycine max*). The resulting soil ecosystem restoration due to the implementation of these good agricultural practices are herewith reported on and discussed. Two reference sites, a conventional agriculture (Conv) farmland (VConv) and a natural veld area (NVV) offered respective disturbed and undisturbed baselines for comparison. The VConv farmland is annually rotated with a cash crop (maize or soybean), while NVV is an adjacent undisturbed natural veld area representing a pristine environment. In this chapter, all farmlands and the natural veld area will be referred to as treatments. The nematode-based indices (NBIs) and selected soil health bioindicators, namely soil respiration (SR), active carbon (AC), and percentage total organic matter (% OM) were used to demonstrate soil ecosystem restoration in farmlands under CA. The effect of abiotic soil factors on ecosystem functioning was further studied to increase understanding of the influence of abiotic soil factors on soil biological processes, which are ultimately responsible for the efficient functioning of soil ecosystems (Vincent *et al.*, 2018).

4.2 Soil ecosystem health and functioning

Calculated NBIs were used to measure the response of the nematode community to agricultural management since it can provide information on the health and functioning of soil ecosystems in farmlands (Ferris *et al.*, 2004). Abundance values of the identified nematode families and genera, the average amount of nematodes per 200 g of soil, and the 10 most prevalent taxa from each sampling site are provided in Table S4.1a, b, and c (supplementary material). Values of NBIs are presented in Table 4.1, while values of the nematode metabolic footprints (NMF) namely the Enrichment, Structure, Bacterivore, Fungivore, Herbivore, Omnivore, and Predator footprints are provided in Table 4.2.

Table 4.1: Mean ± standard error of nematode-based index values (MI 2-5 = Maturity index 2-5, EI = Enrichment index, SI = Structure index, BI = Basal index, and CI = Channel index) of three conservation agriculture farmlands (VCA1-3), a conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede. Based on factorial analysis, common superscript indicates no significant differences (p<0.05) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling Interval					Second Sampling Interval							
Index	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**Effect**	**F ratio**	**p-value**
MI 2-5	^{ab}2,52± 0,09	^{a}2,52± 0,07	^{ab}2,68± 0,07	^{c}2,28± 0,06	^{b}2,80± 0,16	^{ab}2,59± 0,10	^{a}2,45± 0,07	^{ab}2,49± 0,05	^{c}2,15± 0,03	^{b}2,72± 0,09	Treatment	10,97	0,000
											Interval	2,06	0,158
											Treatment* Interval	0,65	0,640
EI	^{a}36,17± 4,10	^{c}67,11± 5,60	^{abc}49,19± 3,69	^{ab}42,08± 2,64	^{ab}37,96± 3,26	^{ab}46,52± 3,62	^{bc}56,12± 4,33	^{ab}40,88± 3,98	^{ab}40,65± 1,62	^{ab}44,73± 4,72	Treatment	10,18	0,000
											Interval	0,09	0,770
											Treatment* Interval	2,81	0,035
SI	^{ab}56,87± 5,96	^{a}56,73± 4,20	^{ab}66,55± 3,76	^{c}37,75± 5,36	^{b}70,02± 6,34	^{ab}60,40± 6,70	^{a}52,28± 5,41	^{ab}56,73± 3,56	^{c}24,68± 4,29	^{b}69,04± 3,73	Treatment	16,33	0,000
											Interval	2,40	0,128
											Treatment* Interval	0,87	0,489
BI	^{a}33,25± 2,86	^{a}22,47± 3,30	^{a}24,76± 2,01	^{b}42,36± 2,81	^{a}24,84± 4,81	^{a}28,72± 3,69	^{a}29,69± 3,76	^{a}32,95± 2,31	^{b}49,63± 2,50	^{a}24,38± 2,74	Treatment	14,55	0,000
											Interval	3,10	0,084
											Treatment* Interval	1,61	0,186
CI	^{a}48,40± 8,19	^{b}13,90± 4,19	^{a}35,21± 6,97	^{ab}38,87± 5,83	^{a}51,90± 13,44	^{a}66,71± 12,46	^{b}42,31± 5,48	^{a}72,13± 9,93	^{ab}65,49± 7,07	^{a}75,79± 9,54	Treatment	4,79	0,002
											Interval	23,29	0,000
											Treatment* Interval	0,30	0,877

4.2.1 Soil ecosystem conditions under conservation agriculture

The Maturity index 2-5 (MI 2-5) indicates changes in the soil ecosystem over long periods of time quantifying structural complexity, development, and succession in the soil food web and was used as an overall measure of soil ecosystem health in this study (Bongers & Ferris, 1999; Maina *et al.*, 2020; Neher, 2001; Wu *et al.*, 2010). No significant interaction ($p<0.05$) occurred for Treatments*Sampling Intervals (Table 4.1). The three CA farmlands presented MI 2-5 values between those of the undisturbed reference site (NVV) and the disturbed reference site (VConv) during both sampling intervals for which NVV presented the highest and VConv the lowest. No significant differences were reported for the MI 2-5 among the three CA farmlands for the two sampling intervals. A significant difference did, however, occur for the MI 2-5 of the CA farmlands compared to that of the VConv farmland reference site for each of the sampling intervals. The CA farmlands VCA1 and VCA3 did not differ significantly from NVV, but VCA2 presented a significant difference from the NVV reference site during both sampling intervals as was evident for the VConv farmland reference site.

A low MI 2-5 for VConv indicated reduced soil ecosystem health and impaired soil processes that resulted due to soil ecosystem degradation over time (Salamun *et al.*, 2017). This might likely have occurred through the use of high amounts of inorganic fertilizer on VConv, which typically causes a reduced MI 2-5 (Maina *et al.*, 2020). Furthermore, continuous soil disturbance associated with Conv practices is also suggested to have most probably contributed towards its lower MI 2-5 as specific soil management practices strongly influence the soil nematode community through modification of nematode abundance (Lazcano *et al.*, 2021). The opposite is consequently true for NVV, which presented the overall highest MI 2-5 over both sampling intervals explained by natural veld areas not being subject to continuous physical soil disturbance or fertilizer input.

Increased MI 2-5 values are therefore characteristic of a less disturbed soil ecosystem (Salamun *et al.*, 2017) and such environments show gradual increases in maturity and stability of the soil ecosystem (Salamun *et al.*, 2014). The significant difference among the MI 2-5 values of the three CA farmlands and the VConv farmland reference site might subsequently be due to the implementation of CA practices on these farmlands, which started seven years before when this study was undertaken. Minimal soil disturbance results from the implementation of CA and the latter are consequently classified as a less disruptive agricultural management practice (Page *et al.*, 2020; Sanaullah *et al.*, 2020). The MI 2-5 values of the CA farmlands were, however, similar to that of NVV, indicating that the improvement of soil ecosystem stability as inferred by increased MI2-5 values represents long-term recovery of the CA farmlands towards the degree of restoration seen in NVV. The implemented CA practices promoted soil ecosystem recovery over time, where continued Conv practices disturbed the soil ecosystem preventing its active recovery (Sanaullah *et al.*, 2020; Sithole *et al.*, 2017) as was evident in the latter treatment having significantly lower MI 2-5 values compared to the CA farmlands.

However, unlike VCA1 and VCA3 farmlands, VCA2 presented a significant difference in MI 2-5 from the reference site NVV. A possible reason might be that VCA2 was in a different crop rotation cycle than the other CA farmlands (see Table 3.4, Chapter 3: Material and Methods) and that MI 2-5 measurements reflect soil ecosystem changes over a long period of time (Ney *et al.*, 2019). This has been the only difference regarding the management practice since these three farmlands were converted to CA in 2012 and likely offers the best explanation for the difference of VCA2 from VCA1 and VCA3 in terms of MI 2-5 measurements.

4.2.2 Soil food web status in farmlands under conservation agriculture

The nematode faunal analysis plots each treatment in one of four quadrats indicating the status of the soil food web based on the abundance and composition of the soil nematode community (Ferris *et al.*, 2001; Maina *et al.*, 2020) with characteristics of each quadrat being displayed in Figure 4.1 a. The nematode faunal analysis for the first and second sampling intervals at Vrede is presented in Figure 4.1 a and b, respectively. The significant differences ($p<0.05$) among treatments in each sampling interval are reported in Table 4.1. The two sampling intervals did not differ significantly from each other for both the Enrichment (EI) and Structure indices (SI). However, a significant interaction for the Treatments*Sampling Intervals was recorded for the EI. In contrast, no significant interaction was reported among treatments for the SI over the sampling intervals.

During both sampling intervals, the CA and NVV treatments were classified as matured and fertile except for VCA2, which plotted in the maturing and N (nitrogen)-enriched quadrat (Figure 4.1 a and b). Treatment VConv, in turn, was classified as degraded and depleted. During the first sampling interval (Figure 4.1 a), VCA2 presented the highest enrichment and was the only CA farmland that differed significantly from both reference sites, NVV and VConv, in terms of the EI. During the second sampling interval (Figure 4.1 b), no significant differences were observed among the treatments for the EI (Table 4.1). In terms of the SI, during both sampling intervals, the CA farmlands differed significantly from VConv, while VCA2 was the only CA farmland that differed significantly from NVV by presenting a lower SI value. The CA farmlands VCA1 and VCA3 did not differ significantly from NVV, while the reference sites, VConv and NVV differed significantly from each other for both sampling intervals.

The significant interaction that occurred among Treatments*Sampling Intervals for the EI over the two sampling intervals (Table 4.1) infers that changes occurred in terms of the nutrient enrichment levels of the soil prior to planting and during the growing season. Enriched conditions likely occur because of an external resource input, environmental changes, and organism mortality (Odum, 1985; Yogaswara *et al.*, 2021). This results in a flush of microbial activity that increase the amount of enrichment nematodes (bacterivores) in the soil food web (Ferris *et al.*, 2001). The lack of significant interaction between Treatments*Sampling Intervals for the SI over the two sampling intervals suggests that seasonal variation did not substantially affect the structure of the soil ecosystem treatments over the two sampling intervals. This is likely because stability in terms of soil ecosystem structure is an indication of progressive recovery (Ferris *et al.*, 2001) and is a slow responding component as it is based on the occurrence of more stable nematode taxa (Maina *et al.*, 2020).

It was found by Zhang *et al.* (2019) that specific tillage practices rather than the influence of the growing season impacted soil nematode community structure. The consistent SI values over the two sampling intervals (Table 4.1) may therefore be ascribed to the continuous implementation of the respective tillage practices associated with the CA and Conv treatments. The CA farmlands VCA1 and VCA3, along with the undisturbed NVV reference site, plotted in the matured and fertile quadrat (Figure 4.1 a and b). Treatments that plot in this quadrat is typical of a stable environment with sufficient enrichment levels to sustain soil organisms and which is highly structured in terms of the occurrence of sensitive nematodes (Ferris *et al.*, 2001; Zhang *et al.*, 2015a). The increased SI values as observed in these treatments are indicative of a complex nematode community structure consisting of multiple trophic links existing in the soil food web (Ferris, 2010; Maina *et al.*, 2020). Natural grasslands or veld areas usually plot in the matured and fertile quadrat portraying high SI values and lower primary productivity (Ferris *et al.*, 2001; Girgan *et al.*, 2020) as was evident for NVV for both sampling intervals in this study.

The lack of significant differences among the two CA farmlands VCA1 and VCA3, and the undisturbed reference site NVV, indicated a degree of similarity among the ecosystem structures of these treatments. This may possibly be ascribed to the higher vegetation diversity typically found in natural veld areas and CA farmlands under cover cropping (Maina *et al.*, 2020). Prior to the first sampling interval (Figure 4.1 a) the CA farmlands were under winter cover crop (WCC) cultivation and during the second sampling interval under summer cover crop (SCC) cultivation. Higher established vegetation diversity provides a wider range of available food resources to a multitude of organisms (Fangjiao *et al.*, 2021; Neher, 2010; Salamun *et al.*, 2017) and the occurrence of mixed plant species in these soil ecosystems is suggested to have resulted in increased development and complexity of the soil ecosystems over the two sampling intervals.

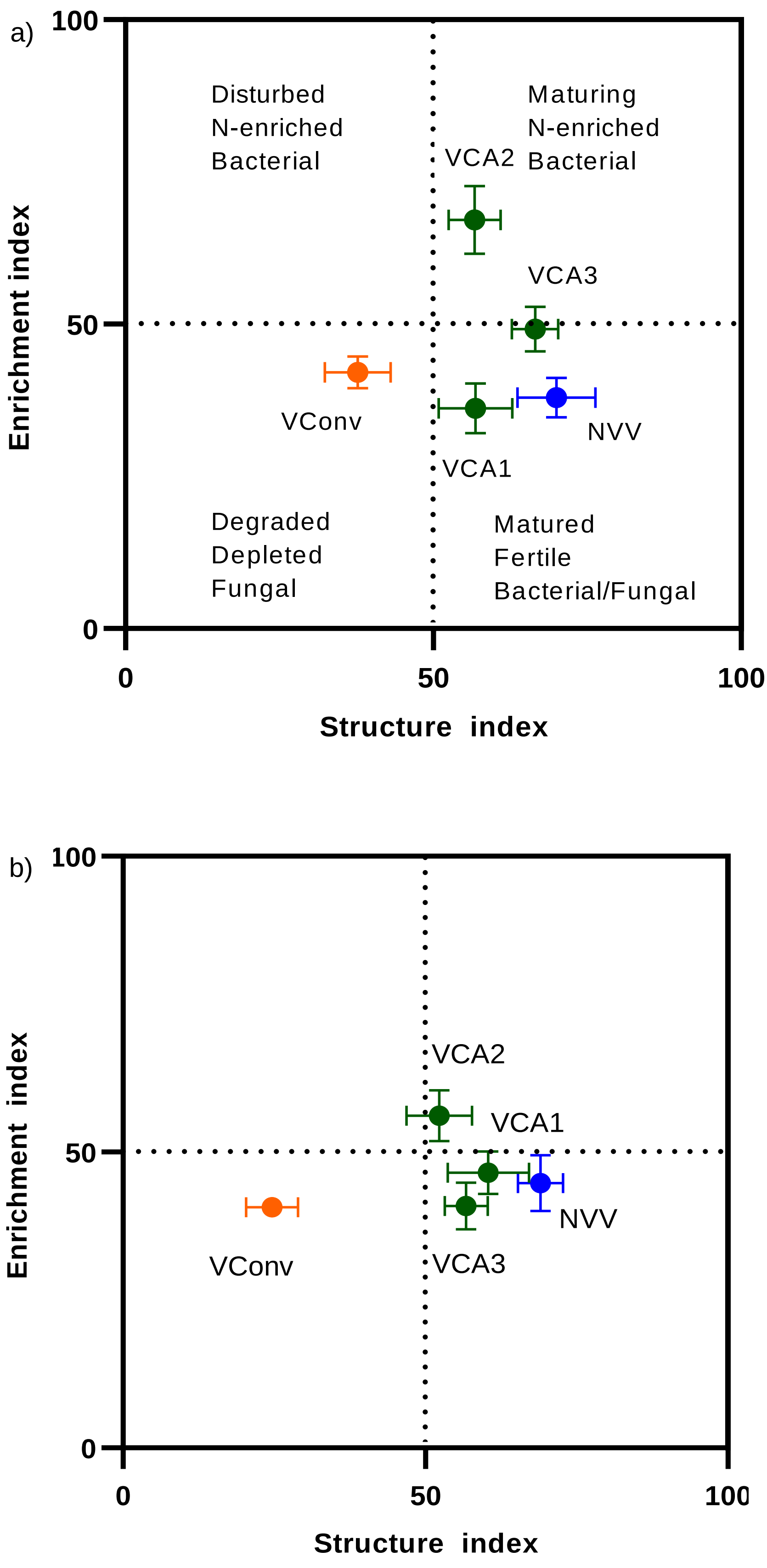


Figure 4.1: The nematode faunal analysis (error bars denote standard error of the mean; n = 6) for three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and one natural veld area/reference site (NVV), in Vrede during the first (a), and second (b) sampling intervals.

Opposed to the other CA farmlands, VCA2 plotted in the maturing and N enriched quadrat during both sampling intervals (Figure 4.1 a and b). These enriched conditions may be ascribed to VCA2's different crop rotation pattern as rotation practices influence the availability of utilizable resources to soil nematodes (Zhang *et al.*, 2015b). During the second sampling interval (Figure 4.1 b) farmland VCA2 also received fertilizer input as it was under maize cultivation, while the other CA farmlands were under SCC cultivation receiving no additional fertilizer. Soil nematode abundance and diversity is affected by N fertilization that changes conditions in the soil ecosystem and increases N availability consequently impacting the nematode food web (Kou *et al.*, 2020) such as is demonstrated for VCA2 in this study; this contribute towards explaining the difference evident for VCA2 compared to the other two CA farmlands in ecosystem conditions.

The reference site VConv farmland, which differed significantly from all other treatments, sets the baseline for a stressed environment that is in a degraded and depleted state. The low soil ecosystem structure of this treatment is typical of a Conv farmland. The management practices implemented at VConv is suggested to have had adversely affected the soil ecosystem through physical disturbance and increased nutrient inputs that quickly changes trophic distribution in the soil food web (Cardoso *et al.*, 2013; Du Preez *et al.*, 2018a; Ferris *et al.*, 2001). It presents a viable explanation for the degraded and depleted state of VConv over both sampling intervals (Figure 4.1 a and b). Decreased SI values indicate reduces soil food web complexity and the shortening of food chains in agricultural farmlands (Georgieva *et al.*, 2002; Mohamedova & Lecheva, 2016).

Except for the MI 2-5, EI and SI that are used as accurate indicators of ecosystem restoration, the primary decomposition channels in the soil food web are determined by the Channel index (CI) (Ferris *et al.*, 2001; Sánchez-Moreno *et al.*, 2018). Increased CI values indicate a more fungal dominated pathway while reduced CI values indicate a mainly bacterial dominated decomposition pathway (Thakur *et al.*, 2019).

Significant differences were reported between the treatments and the sampling intervals, but no significant interaction existed for Treatments*Samping Intervals (Table 4.1). The substantial higher CI values for treatments for the second sampling interval indicated changes in primary decomposition in the soil food webs of the treatments over time. During both sampling intervals farmland, VCA2 presented the lowest CI (<50; bacterial dominated decomposition) and did not differ significantly from VConv that also presented a low CI and had a bacterial dominated decomposition pathway during the first sampling interval. The CA farmland VCA2 differed significantly from the other two CA farmlands (VCA1, VCA3), and NVV that presented higher CI values (>50; fungal dominated decomposition). As suggested by Ferris *et al.* (2001), sites with increased EI values (corresponding to VCA2 and VConv for this study) may present decreased CI values representing bacterial dominated decomposition pathways. The initial low precipitation during the winter months prior to the first sampling interval might also have contributed to the overall lower CI in the farmlands during the first sampling interval (Thakur *et al.*, 2019). The increased CI values of most of the treatments during the second sampling interval might be ascribed to changes in the decomposition matter that are supplied to the soil ecosystem (Ferris *et al.*, 2001).

Disturbance in the soil food web is also indicated by the BI as it measures the occurrence of nematode taxa tolerant to perturbation (Ferris *et al.*, 2001; Maina *et al.*, 2021). A significant difference between the treatments was reported with VConv having a higher BI value than the other treatments (Table 4.1). No significant difference, however, existed between the sampling intervals, neither did a significant interaction occur for Treatments*Sampling Intervals. The reference site VConv with the highest BI during both sampling intervals implies that the soil food web of this farmland was continually disturbed, possibly due to Conv practices like physical soil disturbance and the use of increased amounts of inorganic fertilizer (Briar *et al.*, 2007; Singh, 2018). The opposite was, however, true for the farmlands under CA, highlighting the benefits of the implemented CA practices and the undisturbed state of the natural veld undisturbed reference site NVV.

4.2.3 Soil ecosystem functioning under conservation agriculture

The contribution of nematodes to soil ecosystem functioning was represented by the NMF. In Figure 4.2 a and b, the NMFs namely Enrichment, Structure, Herbivore, Fungivore, Bacterivore, Predator, and Omnivore footprints are presented on radial plots for both sampling intervals (Lazcano *et al.*, 2021). These footprints indicate the flow of energy and C into the soil food web and through soil fauna via the respective decomposition channels (Ferris, 2010; Ferris *et al.*, 2001; Maina *et al.*, 2020). Calculated values of footprints and the significant differences ($p<0.05$) among treatments, sampling intervals, and significant interaction for Treatments*Sampling Intervals is presented in Table 4.2.

Significant interactions for Treatments*Sampling Intervals occurred for the majority of the footprints with the exception of the Fungivore, Herbivore, and Predator footprints; only the treatments differed significantly for the latter footprint (Table 4.2). All NMFs except the Fungivore and Herbivore footprints presented significant differences among the treatments for each sampling interval. Nematode metabolic activity in soil ecosystems may be largely variable despite spatial variation in water and nutrient availability and above-ground conditions typically induced through agricultural management (Lazcano *et al.*, 2021; Luo *et al.*, 2021). In this study, a significant interaction was therefore observed between Treatments*Sampling Intervals for four of the seven NMFs over the two sampling intervals confirming that the continuous implementation of the specific agricultural management practices associated with the treatments impacted soil ecosystem functioning over time. Such variation was likewise reported by Sánchez-Moreno *et al.* (2018) where it was found that the NMF varied according to crop types and management practices. In the current study, seasonal variation was another factor that could have contributed towards ecosystem functioning in the farmlands studied.

A similar trend was reported for the Bacterivore (BF) and Enrichment (EF) footprints where farmland VCA2 presented significantly larger BF and EF than the other CA farmlands and NVV during the first sampling interval (Figure 4.2 a and c). Farmlands VCA2 did furthermore not differ significantly from VConv. During the second sampling interval (Figure 4.2 b and d, however, no significant differences were reported among the treatments for these two footprints.

In terms of the Structure footprint (SF), no significant differences were reported during the first sampling interval (Figure 4.2 a and c). During the second sampling interval (Figure 4.2 b and d), however, farmland VCA1 presented the highest SF and differed significantly from VConv that had the lowest SF. This highlighted favourable conditions in the soil food web towards higher cp-value nematode taxa in the CA farmlands and NVV, from which VCA1 did not differ significantly in terms of the SF.

During both sampling intervals (Figure 4.2 a-d) the Predator footprint (PF) of VConv was significantly lower than that of farmland VCA3 and the undisturbed reference site NVV, but not compared to those of farmlands VCA1 and VCA2. In terms of the Omnivore footprint (OF), no significant differences were recorded among the treatments for the first sampling interval, while the reference site VConv farmland differed significantly from farmland VCA1 and reference site NVV, but not from farmlands VCA2 and VCA3.

The EF and BF are related as the latter may be affected by the EF since the application of organic amendments or inorganic fertilizer can directly enhance bacterial populations in the soil, increasing the presence and density of bacterivorous nematodes (Bulluck & Ristaino, 2002; Maina *et al.*, 2020). Similar results were reported by Kou *et al.* (2020) where fertilizer addition increased the biomass C of bacterivorous nematodes. Bacteria and bacterivorous nematodes are responsible for regulating decomposition in agricultural farmlands (Ewald *et al.*, 2020; Neher, 2010) possibly explaining the elevated BF of the CA farmlands with their increased enrichment levels, as opposed to the reference NVV (Ferris *et al.*, 2012b). The reference site VConv farmland are furthermore subject to increased addition of inorganic fertilizer that promotes the abundance and diversity of bacteria in the soil ecosystem (Nguyen *et al.*, 2018), subsequently indicating an increase in enrichment and confirming the increased BF value.

Table 4.2: Mean ± standard error of nematode metabolic footprint values (Enrichment, Structure, Herbivore, Predator, Omnivore, Fungivore, and Bacterivore footprints) of three conservation agriculture farmlands (VCA1-3), a conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede. Based on factorial analysis common superscript indicates no significant differences (p<0.05) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling Interval					**Second Sampling Interval**							
Index	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**Effect**	**F ratio**	**p-value**
Enrichment footprint	^{abc}60,64± 16,43	^{d}427,94± 89,06	^{abc}62,73± 21,19	^{cd}136,46± 30,30	^{a}14,73± 4,12	^{abc}39,47± 11,19	^{bc}87,52± 25,62	^{ab}32,37± 10,76	^{abc}32,03± 5,51	^{ab}24,50± 8,10	Treatment Interval Treatment* Interval	16,13 15,39 4,18	0,000 0,000 0,005
Structure footprint	^{a}150,54± 50,72	^{a}109,00± 23,86	^{a}138,76± 51,38	^{a}127,92± 42,47	^{a}95,11± 36,92	^{a}157,07± 95,74	^{ab}49,27± 11,83	^{ab}56,36± 11,68	^{b}19,32± 8,57	^{a}87,20± 8,11	Treatment Interval Treatment* Interval	2,65 14,58 3,18	0,044 0,000 0,021
Herbivore footprint	^{a}82,40± 15,10	^{a}64,79± 14,52	^{a}74,42± 9,58	^{a}88,31± 25,04	^{a}100,14± 9,89	^{a}72,16± 10,36	^{a}65,28± 11,67	^{a}70,92± 9,58	^{a}112,40± 39,34	^{a}100,69± 20,41	Treatment Interval Treatment* Interval	1,50 0,00 0,17	0,098 0,937 0,055
Predator footprint	^{ab}26,31± 9,34	^{ab}23,79± 2,85	^{a}39,14± 11,56	^{b}20,31± 9,49	^{a}30,05± 8,06	^{ab}16,94± 5,33	^{ab}20,29± 7,89	^{a}28,35± 7,71	^{b}2,03± 1,31	^{a}37,65± 6,98	Treatment Interval Treatment* Interval	3,12 2,02 0,80	0,023 0,162 0,534
Omnivore footprint	^{a}111,80± 46,37	^{a}83,03± 25,13	^{a}90,75± 41,81	^{a}98,79± 36,58	^{a}53,18± 26,29	^{a}135,59± 93,90	^{ab}26,97± 7,33	^{ab}24,01± 8,16	^{b}11,68± 8,66	^{a}35,00± 8,97	Treatment Interval Treatment* Interval	2,81 16,27 3,31	0,035 0,000 0,018
Fungivore footprint	^{a}19,47± 2,30	^{a}12,03± 1,94	^{a}19,38± 4,04	^{a}28,69± 6,61	^{a}15,11± 3,37	^{a}18,03± 3,18	^{a}17,34± 2,78	^{a}14,82± 1,62	^{a}19,65± 3,21	^{a}25,71± 3,45	Treatment Interval Treatment* Interval	2,07 0,01 2,49	0,098 0,937 0,055
Bacterivore footprint	^{bc}113,22± 20,01	^{d}482,34± 92,83	^{abc}105,0± 25,65	^{cd}251,9± 38,12	^{ab}41,93± 5,66	^{ab}62,97± 21,69	^{ab}95,36± 25,31	^{ab}50,99± 11,74	^{ab}60,34± 8,49	^{a}34,96± 8,07	Treatment Interval Treatment* Interval	14,30 42,90 3,10	0,000 0,000 0,023

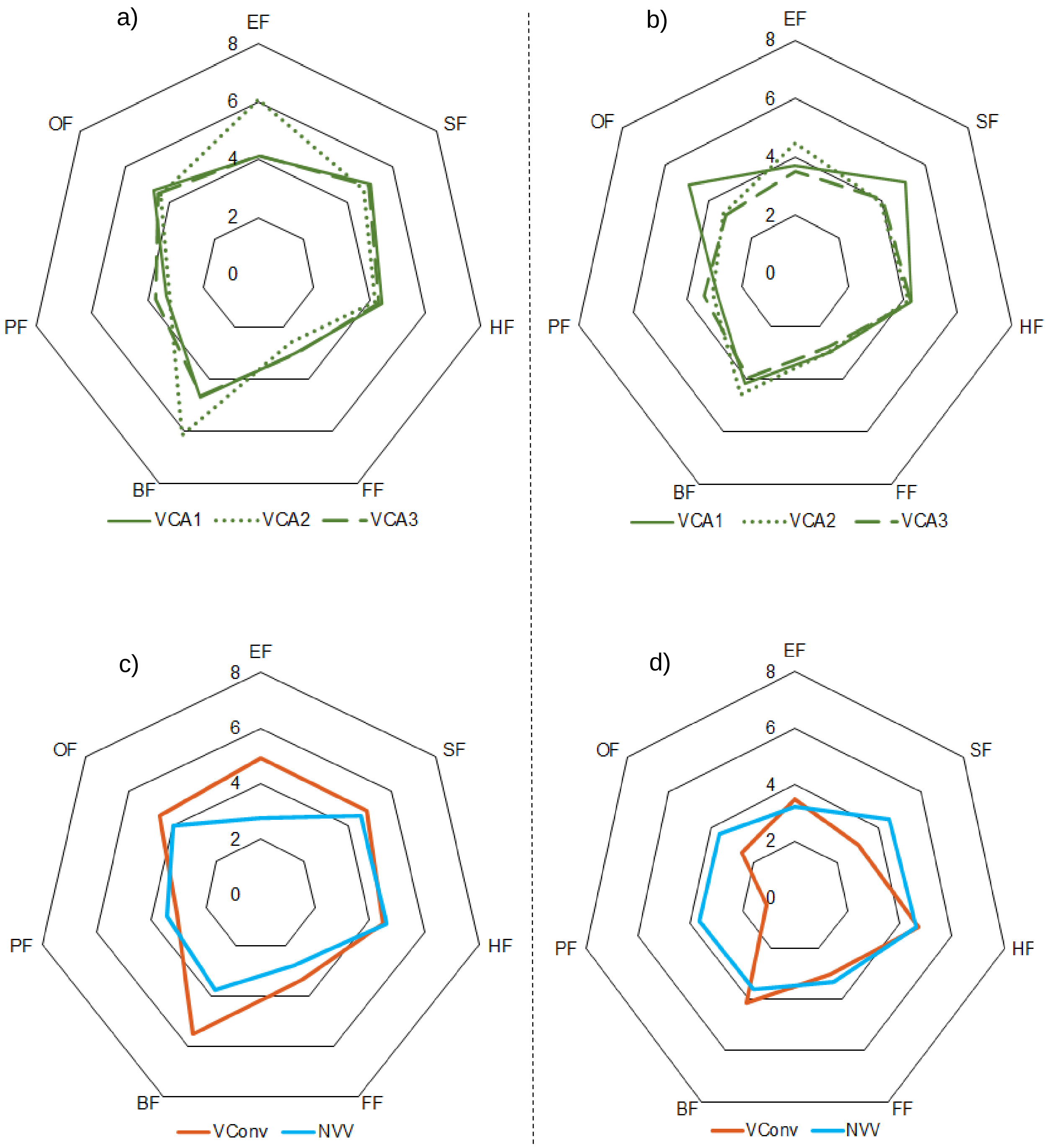


Figure 4.2: The nematode metabolic footprints (EF = Enrichment footprint; SF = Structure footprint; HF = Herbivore footprint; FF = Fungivore footprint; BF = bacterivore footprint; PF = Predator footprint; OF = Omnivore footprint) for three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and one natural veld area/reference site (NVV), in Vrede during the first (a, c), and second (b, d) sampling intervals. Values are the mean of six replicates; n = 6, and data are ln transformed (Lazcano *et al.*, 2021; Sánchez-Moreno *et al.*, 2018).

The significant difference in the BF and EF between treatments VCA2 and NVV during the first sampling interval (Table 4.2) and its non-significant difference from VConv might suggest that the increased enrichment levels in Vconv have also been sourced from inorganic fertilizer application. The second sampling interval occurred during the growing season when plants were at a late stage of crop growth resulting in an environment where the easily assessable nutrients are generally depleted and substrates for microbial communities are recalcitrant (Luo *et al.*, 2021; Wardle *et al.*, 2004). This might possibly explain the lack of significant differences among the treatments for the BF and EF during the second sampling interval.

The cultivation of different plant species furthermore returns different qualities and quantities of resources to the soil ecosystem, affecting soil biota and their associated processes (Wardle *et al.*, 2004). Since VCA2 was under different crop rotation than VCA1 and VCA3, it is likely that reduced seasonal fertilization associated with the cover crop cultivation of the latter two farmlands during the second sampling interval, influenced ecosystem functioning to be in a similar state to that of NVV with mineralization services activity within the soil ecosystem (Maina *et al.*, 2020).

Farmland VCA1 presented the highest OF and SF during the second sampling interval (Figure 4.2 b and d) and are therefore considered to have regulatory functions active in the soil ecosystem (Ferris, 2010; Zhang *et al.*, 2015a). Farmland VCA1 also differed significantly from VConv and the CA practices associated with this farmland is suggested to not disrupt the activity of sensitive nematodes responsible for pest regulation and suppression resulting in increased soil ecosystem functioning (Maina *et al.*, 2020). This might subsequently also be true for the other CA farmlands, which did not differ significantly from VCA1 in either the OF and SF and for VCA3, which presented the largest PF and that differed significantly from VConv. The relative sustained PF in the CA farmlands furthermore indicated that the soil ecosystems under CA are not regressing (opposed to VConv) as it provided and sustained the environment for increased pest regulation and suppression services to be delivered by nematode genera from higher c-p classes (Ferris, 2010; Maina *et al.*, 2020; Sánchez-Moreno *et al.*, 2018).

These findings highlighted the inability of the soil ecosystem of VConv to harbour nematodes that are responsible for the more advanced soil ecosystem functions that are established through nematodes belonging to higher c-p classes (Ferris, 2010). The opposite was, however, true where a lack of significant differences between the CA farmlands, and the undisturbed NVV reference site continually occurred, especially during the second sampling interval (Table 4.2). The SF reduction of the reference site VConv farmland may likely be ascribed to the specific tillage practices associated with this treatment (Ewald *et al.*, 2020; Zhong *et al.*, 2017). This might be the case as predatory nematodes that occur in higher trophic levels are very sensitive to soil disturbance e.g., fertilizer application and tillage while requiring increased time to establish in an environment as opposed to bacterivores and fungivores that can adapt and establish rapidly (Bulluck & Ristaino, 2002; Ferris *et al.*, 2001; Maina *et al.*, 2020).

4.3 Other bioindicators of soil ecosystem health

Selected soil health bioindicators were implemented in this study as practical tools for monitoring biological soil processes that quantify soil ecosystem health and functioning as a result of agricultural management practices (Caixeta *et al.*, 2016). Additional to the NBIs, other bioindicators used in this study to measure soil ecosystem restoration included AC, SR, and % OM (Table 4.3).

In terms of SR, significant differences ($p<0.05$) were reported among the treatments as well as significant interaction for Treatments*Sampling intervals. Treatment NVV was the only treatment that differed significantly from all other treatments by presenting the overall highest SR rate over both sampling intervals. The same trend was reported for AC where NVV presented the overall highest values and differed significantly from all other treatments. The reference site VConv, however, represented increased AC content over the CA farmlands during the first sampling interval and partially during the second sampling interval. The treatments and the two sampling intervals differed significantly from each other, but no significant interaction in terms of the AC content between Treatments*Sampling Intervals was reported. During both sampling intervals treatment NVV furthermore contained the highest % OM and differed significantly from all other treatments. No significant differences between sampling intervals and no interaction for Treatments*Sampling intervals were reported for the % OM.

The established natural veld (i.e., NVV) undergoes no fertilizer additions and is largely undisturbed. The only possible disturbance exerted on this treatment is its annual or biannual grazing. Grazing is known to affect the soil ecosystem physically and chemically through the specific grazing practice (Wang *et al.*, 2019). The occurrence of established vegetation furthermore plays an important role in maintaining SR (Cardoso *et al.*, 2013) possibly explaining its increased SR rate over the other treatments. Furthermore, the CA farmlands and VConv consistently presented lower SR than NVV, which could possibly be explained by Chaplot *et al.* (2016) and Ramirez *et al.* (2010) who reported that the addition of inorganic N fertilizer to the soil ecosystem can impede SR in agricultural soils.

As for the SR results, the treatment containing the highest amount of AC was also the undisturbed reference treatment, NVV. It is known that high amounts AC in undisturbed sites serve as a viable food source for soil organisms that can subsequently contribute to increased SR values (Maron *et al.*, 2018). In a study by Ghosh *et al.* (2019a) soil collected from undisturbed forest areas presented a much larger initial amount of AC as opposed to soils that have been under long-term cropping. Furthermore, Sprunger *et al.* (2019) found that established vegetation (perenniality) largely impacts AC, highlighting the capacity of systems under reduced disturbance to accumulate C in stable AC pools. These findings likely support the increased AC content in the undisturbed NVV reference site included in this study. Increased microbial biomass C associated with surface cover furthermore results in a larger AC pool in these soil ecosystems (Kumawat *et al.*, 2020). By contrast, it has been reported that tillage redistributes C to deeper parts of the soil while mineralizing the fractions of AC (Cooper *et al.*, 2016), which may explain the lower AC content of the studied farmlands investigated in this study.

An increase in AC content may also result from the limited soil disturbance achieved through reduced tillage (Cooper *et al.*, 2016). Residue retention due to zero tillage practices also resulted in significant increases in AC content in a study by Kumawat *et al.* (2020) and by Dou *et al.* (2008). The CA farmlands in this study, however, presented lower AC content, which might be explained by their increased SR, since AC serves as a direct food source, supplying energy to respiring organisms (Bongiorno *et al.*, 2019b).

The NVV treatment contained the highest % OM and differed significantly from all the other treatments for both sampling intervals. This treatment was mainly undisturbed and are likely subject to the establishment of permanent soil cover that provides continuous OM input (Sprunger *et al.*, 2019). Opposite to that, the VConv farmland is subject to heavy soil disturbance, which might result in high OM loss. This occurs as tillage increases CO_2 evolution in the soil ecosystem through the sudden introduction of O_2 into the soil, faster turnover of microbial biomass, and enhanced organic C oxidation to CO_2 in Conv systems (Wulanningtyas *et al.*, 2021). Therefore, likely as a result of the implemented CA practices (minimum soil disturbance and permanent soil cover), the CA farmlands displayed decreased rates of OM decomposition (Peixoto *et al.*, 2020; Wulanningtyas *et al.*, 2021).

Considering the low soil organic C content of agricultural soils in South Africa (Swanepoel *et al.*, 2016) the CA farmlands containing cover crops presented a higher % OM than the VConv farmland reference site. The cultivation of cover crops and the presence of their residues may possibly increase the % OM in these soils (Adetunji *et al.*, 2020). This is an important factor as increased OM improves soil ecosystem functions, e.g., nutrient cycling (Adetunji *et al.*, 2020), indicating increased soil ecosystem health (Williams *et al.*, 2020). In terms of the AC, SR and % OM bioindicators, the CA farmlands still, however, differ significantly from NVV but are progressing towards ecosystem health and functional restoration.

Table 4.3: Mean ± standard error of the measured bioindicators (soil respiration - SR, active carbon - AC, and total percentage organic matter - % OM) of three conservation agriculture farmlands (VCA1-3), a conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede. Based on factorial analysis common superscript indicates no significant differences (p<0.05) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling Interval					**Second Sampling Interval**							
Bioindicator	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**Effect**	**F ratio**	**p-value**
SR (ppm)	^{a}66,2± 1,80	^{a}64,43± 2,39	^{a}87,96± 9,68	^{a}81,84± 5,08	^{b}147,68± 8,25	^{a}81,23± 5,04	^{a}81,54± 4,15	^{a}70,46± 2,31	^{a}62,37± 1,15	^{b}159,01± 10,71	Treatment Interval Treatment* Interval	69,42 0,12 4,60	0,000 0,735 0,003
AC (mg/kg)	^{a}480,80± 38,65	^{a}461,40± 74,05	^{a}468,64± 27,68	^{a}510,91± 36,84	^{b}818,65± 63,65	^{a}375,72± 26,49	^{a}412,38± 14,55	^{a}271,07± 20,07	^{a}324,17± 12,89	^{b}590,48± 27,26	Treatment Interval Treatment* Interval	23,00 38,21 1,78	0,000 0,000 0,148
% OM	^{b}11,65± 0,30	^{b}11,32± 0,23	^{a}10,18± 0,23	^{a}9,70± 0,04	^{c}14,50± 0,20	^{b}11,45± 0,25	^{b}11,03± 0,29	^{a}10,13± 0,30	^{a}9,75± 0,19	^{c}13,92± 0,22	Treatment Interval Treatment* Interval	110,77 2,05 0,54	0,000 0,159 0,710

4.4 The effect of abiotic soil factors on soil ecosystem health and functioning

It is necessary to consider the potential influence of the abiotic environment on soils when studying the restoration of soil ecosystem functioning (Vincent *et al.*, 2018). Identifying patterns among the various abiotic drivers of biological processes in the soil substantially contributes to soil ecosystem studies (Yang *et al.*, 2020). Soil abiotic parameters measured as part of this study are presented in Table S4.2 (supplementary material). Multivariate redundancy analyses (RDAs) were performed to investigate the effects of soil abiotic parameters (explanatory variables) on the soil biological component (response variables) and to identify key abiotic drivers of soil ecosystem restoration. Redundancy analyses are illustrated on biplots in Figure 4.3 a and b for the two sampling intervals, respectively.

During the first sampling interval (Figure 4.3 a), the explanatory variables accounted for 56 % of the variation in the measured response variables where 35.8 % ($p<0.05$) of variation was illustrated on axis 1 and 20.2 % ($p<0.05$) on axis 2. Inorganic N presented a strong negative correlation with the SI and MI 2-5, while the NVV treatment was positively correlated with the bioindicators AC, SR, % OM, as well as the CI, MI 2-5, and SI. The CA farmlands were furthermore positively correlated with the EI, MI 2-5, and SI and negatively correlated with the CI. Furthermore, the heavy metals Ni and Cu were positively correlated with the CA farmlands and EI, while presenting negative correlations with the undisturbed reference site NVV, as well as AC, SR, and % OM. Considering the observed variation in response variables, the treatments explained 57 % ($p<0.05$) of the observed variation, inorganic N explained 9.5 % ($p<0.05$), and Ni and Cu explained 6.6 % ($p<0.05$) and 4.2 % ($p<0.05$), respectively.

During the second sampling interval (Figure 4.3 b), the explanatory variables accounted for 54 % of the variation in the measured response variables, where 45 % ($p<0.05$) of variation was explained on axis 1 and 9 % ($p<0.05$) on axis 2. A positive correlation existed between pH and the majority of the bioindicators (AC, EI, MI 2-5, SI, SR, and % OM). The undisturbed treatment NVV was again positively correlated with the bioindicators AC, EI, CI, MI 2-5, SI, SR, and % OM. The CI, MI 2-5, SI, pH, and Co were furthermore positively correlated with the CA farmlands. In this analysis, the treatments were responsible for explaining 71 % ($p<0.05$) of the observed variation in response variables illustrated on the biplot. Soil pH furthermore explained 5.7 % ($p<0.05$) of variation, and Co, 3.5 % ($p<0.05$).

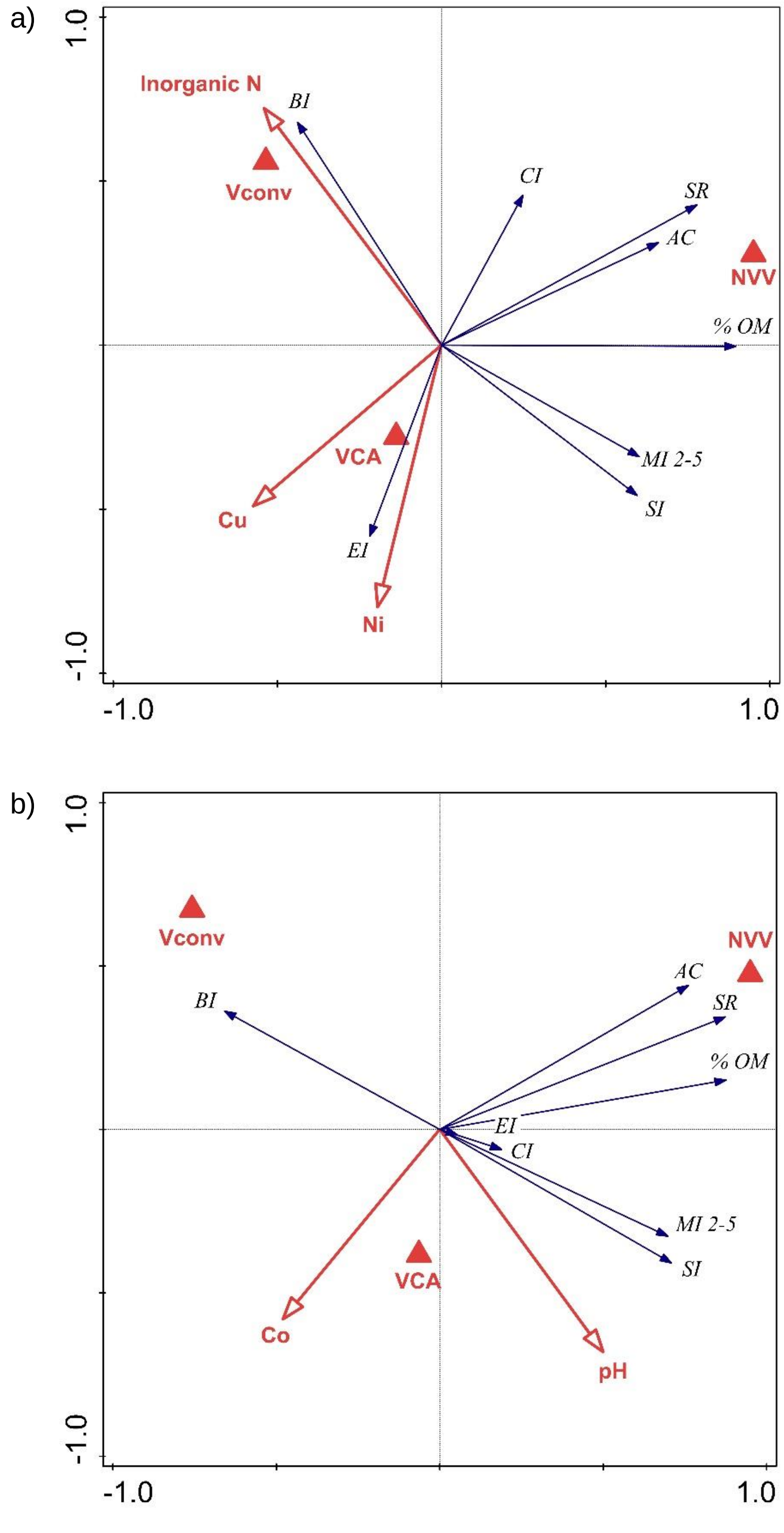


Figure 4.4: Redundancy analysis indicating the correlation between soil abiotic parameters (explanatory variables) and bioindicators (response variables) of soil ecosystem health and functioning for the first sampling interval (a) and second sampling interval (b) in Vrede (VCA = Vrede conservation agriculture farmlands, VConv = Vrede conventional agriculture farmland, NVV = Vrede Natural veld area/reference site, MI 2-5 = Maturity index 2-5, EI = Enrichment index, SI = Structure index, BI = Basal index, CI = Channel index, SR = Soil respiration, AC = Active carbon, % OM = Percentage total organic carbon).

It is evident from the RDAs that the treatments were associated with the various abiotic parameters and bioindicators. The disturbed reference site (VConv) was negatively correlated with the SI and MI 2-5 which agrees with the findings of Lenz and Eisenbeis (2000) and Yogaswara *et al.* (2021) that indicated reduced soil ecosystem health in farmlands under Conv. A negative correlation between VConv and the bioindicators AC, SR, and % OM existed while VConv was also positively correlated with increased inorganic N levels (see Section 4.4.1). The impact of inorganic N is therefore highlighted as it adversely impacted the soil ecosystem health and functioning of VConv (Singh, 2018).

The continuous positive correlation of NVV to the bioindicators indicated increased soil health and soil ecosystem health and functioning, as is evident in undisturbed soils (Williams *et al.*, 2020). The latter author also found that undisturbed, natural areas consistently displayed significant differences from agricultural farmlands as it continuously presents a positive relationship to bioindicators of soil health. In this study, the CA farmlands did not present increased AC, SR, and OM levels as seen for NVV, but were positively correlated with the MI 2-5 and SI. Even though in this study the CA farmlands were not as strongly associated with the measured bioindicators as was evident for the undisturbed NVV reference site, they did present a negative correlation with VConv indicating progression towards increased soil health and restored soil ecosystem function.

The CA farmlands further presented a positive correlation with increased soil pH (see Section 4.4.2) which evidently benefitted soil ecosystem health and functioning. A further positive correlation existed between the CA farmlands and certain heavy metals (see Section 4.4.3) that may possibly be related to the use of synthetic agrochemicals in CA systems (Sanaullah *et al.*, 2020).

4.4.1 The correlation between the inorganic nitrogen and the Basal index

The soil food web of VConv responded to fertilizer addition as basal resources in the soil ecosystem is extensively altered when fertilizer inputs rapidly supply nutrients impacting soil biota (Lazcano *et al.*, 2021). Nematodes indicative of the BI survive under adverse conditions with limited resources or high levels of contamination (Ferris *et al.*, 2001). The BI is representative of nematode taxa that are tolerant to stress and disturbance (Maina *et al.*, 2020). The predominant occurrence of this group of nematodes highlights the state of the soil ecosystem of VConv as it is positively correlated with the BI during both sampling intervals (Figure 4.3 a and b). This farmland receives higher amounts of seasonal fertilizer than the CA farmlands and increased inorganic N addition to the soil ecosystem adversely impacts its structure and functioning (Cheng *et al.*, 2021) as inorganic N addition to soils negatively affects soil nematodes (Wei *et al.*, 2012).

4.4.2 The correlation between soil pH and soil bioindicators

Soil pH was found by Kitagami *et al.* (2020) to be a key factor in determining nematode community composition and was also identified by Salamun *et al.* (2017) as an explanatory variable for nematode community structure. As evident here, a higher pH correlated positively with the NBIs and SR, AC and % OM. Soil pH substantially affects the soil biota that is responsible for the maintenance of various soil processes (Nguyen *et al.*, 2018; Yang *et al.*, 2016). During the second sampling interval (Figure 4.3 b) a higher pH might have enabled higher SR, AC content, % OM, and increased SI and MI 2-5 values in the CA farmlands and the undisturbed NVV reference site. This indicates that the implemented CA practices that are implemented is either buffering acidity or is not causing acidification of the soil ecosystem through the increases in % OM (Duiker & Beegle, 2006) and increased vegetation cover (Salamun *et al.*, 2017). The negative correlation between the BI and the majority of the bioindicators points towards a lower pH associated with the VConv farmland that is known to cause soil ecosystem disturbance (Kitagami *et al.*, 2020). An environment with a decreased pH that is strongly correlated with the BI typically indicates a more disturbed soil ecosystem (Maina *et al.*, 2020). Acidification of agricultural soils characterised by lower pH values is typically the result of the over-application of N fertilizer and is the largest contributor to soil fertility loss (Du Preez & Van Huyssteen, 2020) and therefore, also to reductions in soil ecosystem health and functioning as observed in VConv. In terms of soil ecosystem functioning, soil pH affects the stability of fungivores, predators, and omnivores as they are very sensitive organisms and increased soil pH can decrease soil nematode community stability (Liang *et al.*, 2020).

4.4.3 The correlation between various heavy metals and soil bioindicators

A strong correlation furthermore existed between the CA farmlands and Co, Cu, and Ni. Synthetic agrochemicals contain, among others, Co, Cu, and Ni as active ingredients (Zoffoli *et al.*, 2013). When synthetic agrochemicals are extensively used for a prolonged period it may lead to the possible build-up of heavy metals in agricultural soils with the subsequent entry of such metals into the soil food web (Naccarato *et al.*, 2020). These heavy metals enter the soil ecosystem through the application of e.g., inorganic fertilizer, pesticides, and manure application (Bai *et al.*, 2011). Furthermore, the longer agricultural farmlands are under anthropogenic management, the higher the probability of synthetic agrochemical discharge resulting in higher heavy metal concentrations (Alengebawy *et al.*, 2021).

The correlation of Ni and Cu with the EI during the first sampling interval (Figure 4.3 a) possibly suggest that the prominent presence of these metals might be sourced from the application of inorganic fertilizer. Inorganic fertilizers are the major source of enrichment in soil ecosystems causing increased EI values (Maina *et al.*, 2020). Furthermore, the metals Cu and Ni presented a strong negative correlation with AC, SR, % OM, and subsequently with NVV, which is known for its high level of soil ecosystem functioning. These elements are heavy metal contaminants (Aislabie *et al.*, 2013), which will most likely remain in the soil ecosystem for a long time, impacting soil biota and therefore adversely affecting ecosystem functioning (Van-Zwieten *et al.*, 2004).

The heavy metal contaminant Co (Aislabie *et al.*, 2013) has been correlated negatively with the bioindicators AC, SR, and % OM, but was positively correlated with the CA farmlands during the second sampling interval (Figure 4.3 b). A rapid accumulation of Co generally occurs through organic soil amendments and is removed through plant uptake or erosion over time (Bhattacharyya *et al.*, 2008). It also occurs as an active ingredient in various pesticides including fungicides and herbicides (Zoffoli *et al.*, 2013). The accumulation of heavy metals like Co is a major culprit in causing soil degradation (Jaiswal *et al.*, 2018; Mahey *et al.*, 2020). The uptake of Co by plants are, however, restricted in the presence of a higher soil pH and high amounts of OM (Luo *et al.*, 2010). During this sampling interval, an increased pH was reported in the CA farmlands, possibly suggesting that Co are not phytotoxic in these farmlands. It is, however, an environmental contaminant and its presence adversely affect soil biota's ability to promote soil ecosystem functioning, as increased Co concentrations impose harmful effects on soil biota, affecting their population diversity, size, and activity (Ashraf & Ali, 2007; Jaiswal *et al.*, 2018). This supports the negative correlation between Co and the bioindicators of soil health used in this study. If Co concentrations may be reduced in the CA farmlands, a possible increase in soil health can be expected especially in terms of AC, SR, and % OM.

The strong, negative correlation between Cu and SR was further investigated using a linear regression model ($R^2 = 0.61$; $p<0{,}001$) as illustrated in Figure 4.5. It has been reported in literature that high Cu concentrations in the soil ecosystem reduce soil microbial activity, subsequently reducing SR and adversely affecting soil fertility (Dumestre *et al.*, 1999; Van-Zwieten *et al.*, 2004). Aoyama and Nagumo (1997) furthermore reported that the inhibition of soil respiration is related to soil Cu levels. Similarly, in this study, the CA farmlands and VConv are positively correlated with Cu and consequently also presented lower levels of SR. The presence of metals like Cu and Ni primarily reduces SR through restrictions like extracellular enzyme inhibition in soil biota (Kandeler *et al.*, 2000; Morawska-Płoskonka & Niklińska, 2013). These heavy metal contaminants (Cu and Ni) in the soil ecosystem is currently inhibiting SR, but due to the higher pH they are likely not phototoxic (Korthals *et al.*, 1996) as the bioavailability of such contaminants is largely controlled by pH and other soil abiotic properties (Alva *et al.*, 2000). The high Cu concentration in the CA farmland may be sourced from the use of fertilizer that caused its accumulation in soil over time (Shahbazi *et al.*, 2018; Wang *et al.*, 2020). Additionally, many widely used fungicides also contain Cu and its continued use can be a major source of elevated Cu levels in soil ecosystems (Al-Wabel *et al.*, 2017).

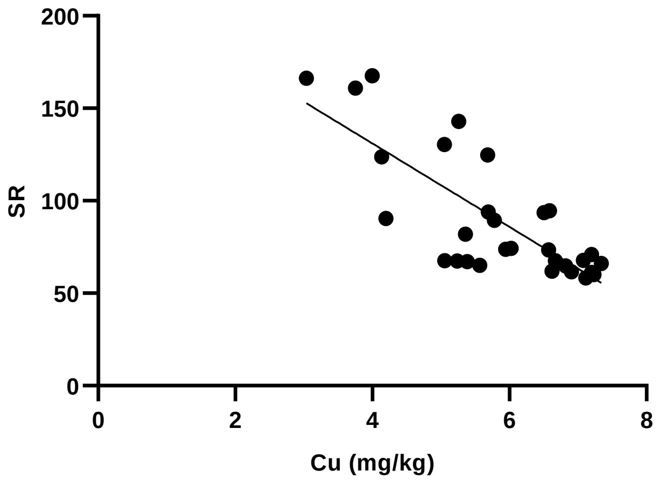


Figure 4.5: A linear regression model indicating the strong negative correlation between Cu and soil respiration during the first sampling interval in Vrede.

4.5 Conclusion

The CA farmlands presented elevated soil ecosystem functioning as measured against the Conv reference site. Furthermore, the CA farmlands presented relatively low soil ecosystem disturbance, except for VCA2 that portrayed increased disturbance possibly induced by the application of inorganic N fertilizer (Zhang *et al.*, 2017). The other CA farmlands were, however, classified as matured and fertile along with the undisturbed NVV reference site, while VConv was classified as a degraded and depleted environment. For the bioindicators of soil health selected for this study (i.e., AC, SR, and % OM), treatment NVV presented the overall highest values, portraying healthier levels of soil ecosystem health (Nunes *et al.*, 2018). Results for the CA farmlands were, however, variable and their relationship towards the selected soil health bioindicators were better portrayed in the RDAs. The CA farmlands presented positive correlations to especially the SI and MI 2-5. It is therefore suggested that the implementation of CA practices likely promote soil ecosystem restoration, with CA farmlands under no-tillage and cover cropping actively progressing toward restored soil ecosystem health (Acharya *et al.*, 2019; Wulanningtyas *et al.*, 2021). This is crucial for sustainable crop production and promoting food security. Emphasis is furthermore placed on the prudent use of synthetic agrochemicals as heavy metal accumulation may inhibit active soil ecosystem recovery.

CHAPTER 5: REITZ RESULTS AND DISCUSSION

5

5.1 Introduction

The sustainability of conservation agriculture (CA) is dependent on the implementation of good agricultural practices like reduced fertilizer inputs and well-planned crop rotation (Rusinamhodzi, 2015). The latter is particularly important where nematodes cause problems since a wide range of rotation crops used, e.g., by South African producers in grain production areas, are susceptible to the predominant nematode pests occurring in such soils (Mc Donald *et al.*, 2017). Reduced fertilizer usage and crop rotation are the major aspects of CA practices implemented on the Reitz CA farmlands (RCA1-3) that were investigated in this study. The three CA farmlands under cash crop rotation are also under a 15 - 20 % reduction in fertilizer use compared to conventional agriculture (Conv) as communicated by the farmer. The benefits of diverse cash crop rotation and reduction in fertilizer use to the soil ecosystem are reported and discussed in this chapter. The Conv farmland (RConv) served as a disturbed reference site that are under Conv practices, while treatment NVR (natural veld Reitz) offered an undisturbed reference site as it is a natural veld area where ultra-high-density grazing (UHDG) are continually implemented. In this chapter, all farmlands and the NVR will also be referred to as treatments. Nematode-based indices (NBIs) were used to measure the restoration of soil ecosystem health and functioning in farmlands (Shaw *et al.*, 2019; Yeates, 2003). Additionally, the bioindicators soil respiration (SR), active carbon (AC), and percentage total organic matter (% OM) were measured as an indication of the ecosystem health of these farmlands (Van Es & Karlen, 2019). Furthermore, the effect of soil abiotic properties on the health and functioning of soil ecosystems were investigated as the soil physiochemical environment determines the state of soil biota, largely by setting limits to its occurrence and functioning (Kibblewhite *et al.*, 2008).

5.2 Soil ecosystem health and functioning

Abundance values of the identified nematode families and genera, the average amount of nematodes extracted from 200 g of soil, and the 10 most prevalent nematode taxa at each sampling site are provided in Table S5.1a, b, and c (supplementary material). Calculated NBIs are presented in Table 5.1 and nematode metabolic footprints (NMF), namely the Enrichment, Structure, Bacterivore, Fungivore, Herbivore, Omnivore, and Predator footprints, are presented in Table 5.2. A varying soil texture was previously reported among the treatments in Reitz. This was an important consideration taken into account during statistical analyses as abiotic factors like soil texture plays an important role in the occurrence and distribution of soil biota (Erktan *et al.*, 2020; Ronn *et al.*, 1995).

Table 5.1: Mean ± standard error of nematode-based index values (MI 2-5 = Maturity index 2-5, EI = Enrichment index, SI = Structure index, BI = Basal index, and CI = Channel index) of three conservation agriculture farmlands (RCA1-3), a conventional agriculture farmland (RConv), and a natural veld area/reference site (NVR) in Reitz. Based on factorial analysis, common superscript indicates no significant differences ($p<0.05$) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling interval					**Second Sampling interval**							
Index	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**Effect**	**F ratio**	**p-value**
MI 2-5	^{ad}2,18± 0,06	^{abc}2,56± 0,08	^{d}2,04± 0,02	^{abc}2,57± 0,12	^{c}2,90± 0,08	^{abc}2,58± 0,13	^{abc}2,63± 0,13	^{abcd}2,47± 0,10	^{abd}2,24± 0,09	^{bc}2,65± 0,09	Treatment Interval Treatment* Interval	8,90 1,04 6,53	0,000 0,312 0,000
EI	^{a}33,93± 1,93	^{a}38,31± 3,60	^{a}29,42± 3,22	^{a}29,86± 4,23	^{a}36,69± 6,67	^{a}27,64± 3,32	^{a}35,57± 3,96	^{a}32,79± 8,27	^{a}19,70± 5,36	^{a}37,46± 4,54	Treatment Interval Treatment* Interval	2,23 0,97 0,63	0,079 0,329 0,647
SI	^{bd}26,69± 6,59	^{ac}61,78± 4,75	^{d}7,62± 3,44	^{abc}57,42± 6,44	^{a}75,88± 2,82	^{abc}58,46± 8,27	^{a}64,08± 8,83	^{abc}50,13± 10,39	^{bcd}30,76± 9,91	^{a}65,87± 4,91	Treatment Interval Treatment* Interval	11,31 3,16 8,22	0,000 0,082 0,000
BI	^{bcd}52,47± 3,34	^{ab}30,75± 3,49	^{d}66,11± 2,07	^{abc}35,12± 4,63	^{a}19,92± 1,32	^{abc}34,49± 6,16	^{ab}29,69± 6,25	^{abcd}41,94± 10,20	^{cd}55,83± 6,03	^{a}27,21± 2,59	Treatment Interval Treatment* Interval	10,96 0,84 6,11	0,000 0,364 0,000
CI	^{a}81,90± 7,28	^{a}60,56± 13,71	^{a}64,58± 9,21	^{a}79,00± 9,88	^{a}86,01± 13,99	^{a}80,73± 9,31	^{a}47,88± 14,55	^{a}58,20± 19,09	^{a}100,00± 0,00	^{a}72,23± 13,24	Treatment Interval Treatment* Interval	2,83 0,11 0,63	0,034 0,740 0,644

5.2.1 Condition of the soil ecosystems

For the Maturity index 2-5 (MI 2-5), a significant difference ($p<0.05$) between treatments, as well as a significant interaction, were recorded for Treatments*Sampling Intervals (Table 5.1). During the first sampling interval two of the three CA farmlands, RCA1 and RCA3, presented the lowest MI 2-5 values differing significantly from the undisturbed reference site NVR. The two reference sites (RConv and NVR) did not differ significantly from each other during either of the sampling intervals and no significant differences were reported among any of the treatments during the second sampling interval. During the second sampling interval, however, the three CA farmlands displayed increased MI 2-5 values compared to the first sampling interval, while the MI 2-5 of the two reference sites (RConv and NVR) decreased. The MI 2-5 of the reference site NVR, however, remained the highest, though not significantly higher than the other treatments, for both sampling intervals.

The high MI 2-5 of the undisturbed NVR reference site suggested that its soil food web was less disturbed than those of the other treatments (Table 5.1). Farmlands RCA1 and RCA3 displayed significantly lower MI 2-5 values than that of the NVR treatment, indicating that their soil food webs were more disturbed during the first sampling interval. Crop rotation is practiced on the CA farmlands and has a known beneficial impact on yield production and pest management, but its direct impact on soil biota is not yet clearly understood (D'Acunto *et al.*, 2018; Venter *et al.*, 2016).

The cultivation of winter wheat (*Triticum aestivum*) and sunflower (*Helianthus annuus*) might have therefore possibly affected the MI 2-5 of the CA farmlands. These crops depletes soil moisture throughout the soil profile (Moroke *et al.*, 2005) and soil moisture reductions may affect soil fauna like nematodes (Dube *et al.*, 2015; Rachidi *et al.*, 1993; Song *et al.*, 2016) that are aquatic animals living in the water films around soil particles (Bakonyi *et al.*, 2007; Da Silva *et al.*, 2021). Soil moisture was however not measured in this study and its impact on the MI 2-5 cannot be confirmed. The CA farmlands are, however, under the same cash crop rotation eliminating the impact of differences in crop rotation that might have affected the MI 2-5. Another possible reason for the variation observed among the CA farmlands may be due to the reported differences in soil texture as each might therefore have a unique water holding capacity affecting the community structure of soil fauna (Liu *et al*., 2019). A further example includes more fine-textured soils that may consequently host smaller nematodes than soils with a coarser texture (Liu *et al.*, 2019; Naveed *et al.*, 2016; Sechi *et al.*, 2018) since this may consequently affect the occurrence of specific nematode taxa. The effect of soil texture is further elaborated on in Section 5.4.

Other possible reasons for the high levels of disturbance in the CA farmlands during the first sampling interval may include the allelopathic effect of the cultivation of sunflower and the use of herbicides. Various allelochemicals are derived from sunflower that may impact biological activity in the soil ecosystem (Jabran, 2017). Limited information, however, exists on the direct or indirect effect of allelochemicals on the soil nematode community and the duration of its effect. It was, however, suggested by Wato (2020) that allelochemicals might be effective in managing plant-parasitic nematodes indicating that the presence of allelopathy from higher plants may affect the soil nematode community. Herbicides are furthermore applied to these farmlands and it was indicated by Baghel *et al.* (2020) that plant-parasitic nematodes can be adversely affected by herbicide use as it reduces the growth of weeds or through its direct toxicity to nematodes.

A significant interaction was furthermore reported for the MI 2-5 between Treatments*Sampling Intervals (Table 5.1) and is evident since the CA farmlands presented increased soil ecosystem health during the second sampling interval, which took place during the growing season. The reason for increased soil ecosystem health of the CA farmlands during the growing season is however unclear. It is known that soil ecosystem recovery from disturbance may be a timely process since nematodes belonging to higher trophic levels are not only sensitive to disturbances but has longer generation times, and produce fewer offspring (Ferris *et al.*, 2001; Maina *et al.*, 2020). The opposite was however observed for the CA farmlands where soil ecosystem health as measured by these organisms increased within a season. The reason that the improved soil ecosystem health of the CA farmlands cannot be directly explained might be that the specific parameter(s) that prompted the alleviation from disturbance were not measured in this study.

The RConv farmland reference site however presented decreased soil ecosystem health during the second sampling interval. This reference site farmland was under soybean (*Glycine max*) cultivation and displayed significantly higher MI 2-5 values than CA farmland RCA3, but not the other two CA farmlands (RCA1 and RCA2), during the first sampling interval (Table 5.1). It also did not differ significantly from the undisturbed reference site NVR during both sampling intervals. The cultivation of soybean has shown to benefit the soil ecosystem and to improve soil ecosystem resistance to disturbances (Gao *et al.*, 2017; Nguyen *et al.*, 2020). The RConv farmland reference site was previously under continuous maize monocropping, with the cultivation of soybean for the first time in the growing season prior to the first sampling interval. It received higher amounts of inorganic fertilizer with the cultivation of maize during the second sampling interval, which might have possibly caused the reduction in MI 2-5 as increased fertilization coupled with conventional tillage causes disturbance in the soil ecosystem (Bongers & Ferris, 1999; Yogaswara *et al.*, 2021).

The soil nematode community of natural veld or grassland areas are furthermore usually distinct from agricultural farmlands and dependant on the specific management system (Viketoft *et al.*, 2011; Vink *et al.*, 2020). In this study, the undisturbed reference site NVR were furthermore under the restorative grazing practice of UHDG. The impact of managed grazing practices can become evident after an extended time as beneficial changes to the soil ecosystem are derived from root exudates, vegetation succession, and litterfall (Bardgett *et al.*, 2005; Viketoft *et al.*, 2011). This site presented significantly higher MI 2-5 values than two of the CA farmlands (RCA1 and RCA3) during the first sampling interval (Table 5.1). It presented the overall highest MI 2-5 of all the treatments. Similar results were reported by Salamun *et al.* (2017) where an established grassland also presented the least disturbed soil ecosystem in comparison with areas with less vegetation, such as agricultural farmlands.

5.2.2 Soil food web status

The nematode faunal analysis was used to classify each treatment into one of four quadrats that highlight the status of the soil food web based on the composition of the soil nematode community (Ferris *et al.*, 2001; Lazcano *et al.*, 2021; Maina *et al.*, 2020). The characteristics of each quadrat is presented in Figure 5.1 a. The faunal analysis for the first and second sampling intervals at Reitz is shown in Figure 5.1 a and b, respectively. Significant differences between treatments, as well as a significant interaction ($p<0.05$) for Treatments*Sampling Intervals, were reported for the Structure index (SI), but not for the Enrichment index (EI) (Table 5.1). During the first sampling interval (Figure 5.1 a) the CA farmlands RCA1 and RCA3 plotted in the degraded and depleted quadrat as opposed to RCA2, which plotted in the mature and fertile quadrat along with RConv and NVR. During the second interval (Figure 5.1 b), however, RCA1 and RCA3 shifted toward the matured and fertile quadrat, where treatments NVR and RCA2 remained. The reference site RConv farmland, in turn, shifted and plotted in the degraded and depleted quadrat.

The significant interaction that occurred for (Treatments*Sampling intervals) in terms of the SI, but not the EI (Table 5.1), might indicate that the use of e.g., inorganic fertilizer did not affect the EI of the farmlands over the two sampling intervals. Possible reasons for the occurrence of changes in the SI might include differences in the input of belowground C from the different crops in rotation as the specific plant community may affect soil ecosystem structure and functioning (Philippot *et al.*, 2013; Sprunger *et al.*, 2019). It was furthermore found by Bakonyi *et al.* (2007) that the SI could be affected by minute changes in soil moisture and temperature. Such fluctuations throughout this study were not measured but might have contributed to the reported significant interaction of the SI over the two sampling intervals since a seasonal change occurred.

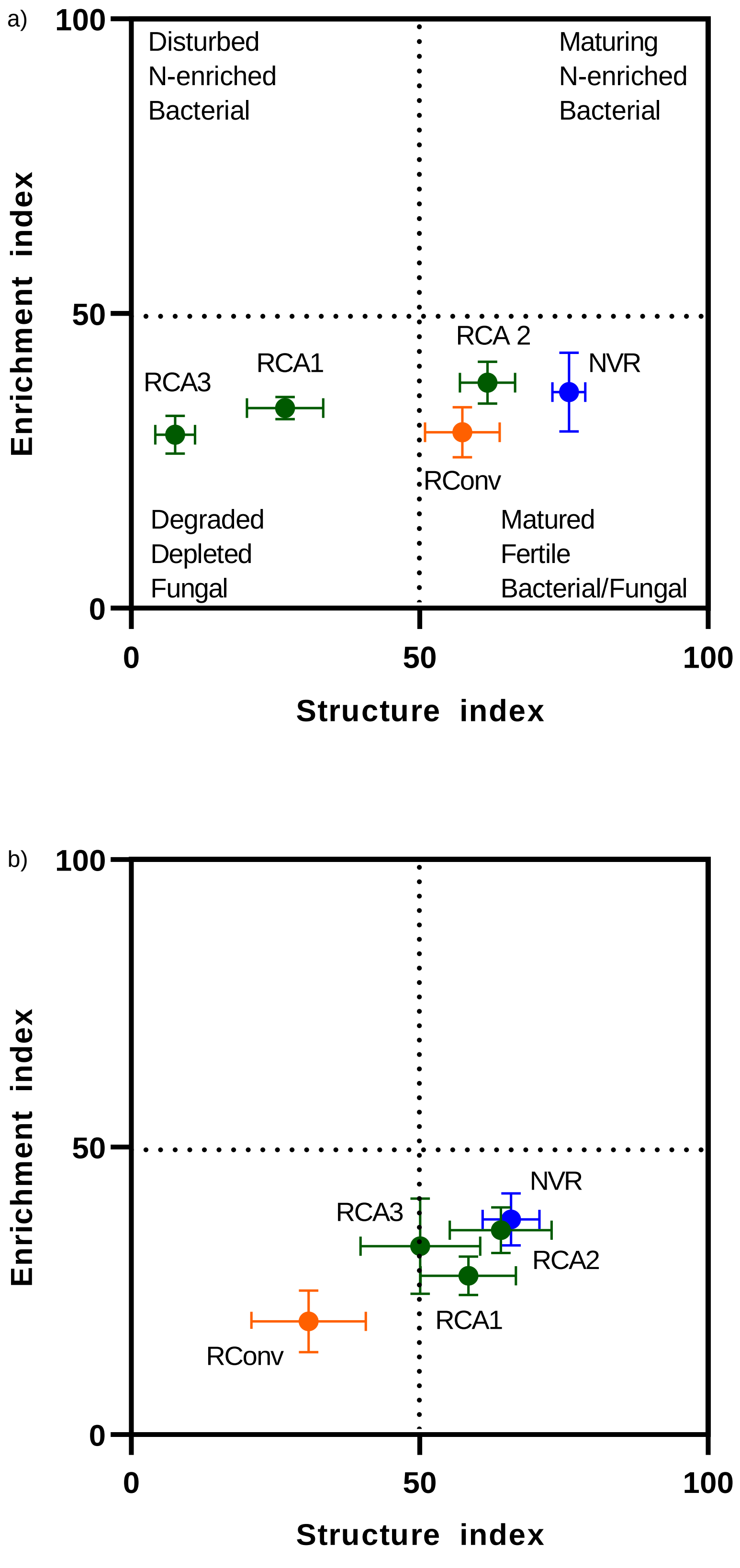


Figure 5.1: The nematode faunal analysis (error bars denote standard error of the mean; n = 6) for three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv), and one natural veld area/reference site (NVR) in Reitz during the first (a) and second (b) sampling intervals.

The average precipitation for the months of March to August was 28 mm with a subsequent increase to 78 mm average for the period from September to February (growing season) (Refer to Chapter 3: Material and Methods). Yan *et al.* (2018) reported a decrease in soil nematode community structure of farmlands due to dry periods. The first sampling interval occurred during the early days of September following the drier winter months. The CA farmlands (RCA1 and RCA3) that plotted in the degraded and depleted quadrat (Figure 5.1 a) is furthermore representative of disturbed areas since they are under agricultural management. Such areas are known to present a decrease in soil ecosystem functions delivered by soil biota especially under the impact of reduced precipitation (Da Silva *et al.*, 2021; Schwarz *et al.*, 2017). This was, however, not displayed by RCA2 that plotted in the matured and fertile quadrat during both sampling intervals. The reason for the higher SI of RCA2 and the significant difference of RCA2 from the other CA farmland (RCA1 and RCA3) during the first sampling interval however remains unknown.

The reference site RConv shifted from the matured and fertile quadrat to the degraded and depleted quadrat during the second sampling interval (Figure 5.1 b). The reason for its classification as matured and fertile during the first sampling interval may be attributed to the inclusion of a rotational legume crop that could have caused an increase in N levels in the soil ecosystem (Sharma *et al.*, 2018). This resultantly may have beneficially impacted soil ecosystem structure and functioning (Cheng *et al.*, 2021). The specific reason for its classification as matured and fertile remains debatable, while its shift toward the degraded and depleted quadrat is what is expected of farmlands under Conv as these tend to be representative of stressed environments (Sithole *et al.*, 2016). The cultivation of maize that occurred during the second sampling interval furthermore required increased N fertilization as communicated by the farmer and such additions may also decrease the occurrence of more stable nematode taxa (Ye *et al.*, 2020) that contributes towards decreased SI measurements (Ferris *et al.*, 2001).

During the second sampling interval (Figure 5.1 b) the CA farmlands RCA1 and RCA3 shifted towards the matured and fertile quadrat in which RCA2 also remained. This represents improvements in terms of increased soil ecosystem structure which may be attributed to the benefits to the soil ecosystem that may be derived from the implementation of CA, which is less disruptive than Conv (Sanaullah *et al.*, 2020).

Treatment NVR were furthermore classified as mature and fertile during both sampling intervals (Figure 5.1 a and b). It was suggested by Savory and Parsons (1980) that grazing practices like UHDG may enhance recovery of the soil food web and soil ecosystems of grasslands (Chaplot *et al.*, 2016). The subsequent reason for this is that livestock flattens the grass during grazing forming a soil cover that together with livestock manure are subject to biological decay that supplies energy to the soil ecosystem (Chaplot *et al.*, 2016; Savory & Parsons, 1980). Appropriate grassland management through practices like UDHG has the potential to restore soil ecosystem functioning and increase its resilience to external pressures (Teague & Kreuter, 2020). Wang *et al.* (2006) reported increased soil ecosystem structure as the numbers of persistent bacterivores, omnivores, and predators increased in grasslands under managed grazing. Similar results were reported in this study as it is possible that UDGH contributed towards benefitting the soil food web by maintaining soil ecosystem structure.

No significant differences were reported among the treatments for the CI for both sampling intervals (Table 5.1). All treatments, except RCA2 during the second sampling interval, presented a CI>50 which is indicative of a fungal dominated decomposition pathway (Ferris *et al.*, 2001; Glavatska *et al.*, 2017). Lower CI values represent faster decomposition and faster nutrient turnover, and the opposite is therefore true for increased CI values (Bongiorno *et al.*, 2019a). The latter authors found that the CI may increase under reduced tillage but opposing results were reported in this study where farmlands under reduced tillage did not necessarily present higher CI values. It may therefore be possible that soil type and the form in which soil nutrients occur, as suggested by Ingham *et al.* (1985) and Ferris and Matute (2003), might have affected the dominant decomposition channel (Maina *et al.*, 2021).

In terms of the BI, which forms part of the nematode faunal analysis (Ferris *et al.*, 2001), a significant difference was reported between the treatments and a significant interaction occurred for Treatments*Sampling Intervals indicating definite variation in soil ecosystem disturbance over time. As shown in Table 5.1, RCA1 and RCA3 presented no significant differences as these two treatments had the highest BI, therefore being the most basal during the first sampling interval (Figure 5.1 a). During the second sampling interval (Figure 5.1 b) no significant differences occurred among the CA farmlands and NVR indicating increased soil food web recovery. Farmland RCA3 presented no significant difference from RConv that had the most disturbed soil ecosystem. Again, no explanation can be given for RCA3 differing significantly from the other two CA farmlands, as indicated for the other NBIs above, in this respect.

5.2.3 Soil ecosystem functioning

Nematode metabolic footprints were used to measure the effect of CA on soil ecosystem functioning and indicates the magnitude of ecosystems functions that are fulfilled by nematodes (Ferris, 2010; Zhang *et al.*, 2015b). The NMFs indicate the flow of energy and C into and through the soil food web via the respective decomposition channels of the dominating trophic groups (Ferris, 2010; Ferris *et al*., 2001; Maina *et al*., 2020). In Figure 5.2 a-d, the NMFs (Enrichment, Structure, Herbivore, Fungivore, Bacterivore, Predator, and Omnivore footprints) for both sampling intervals are presented on radial plots (Lazcano *et al*., 2021). Calculated NMFs and significant differences ($p<0.05$) among treatments, sampling intervals, and significant interactions for Treatments*Sampling Intervals, are presented in Table 5.2.

No significant differences among treatments and no significant interaction were reported for the Enrichment footprint (EF) during both sampling intervals, which indicated that the contribution of enrichment nematodes to soil ecosystem functioning was similar among the different treatments (Table 5.2). Significant differences between treatments and a significant interaction for Treatments*Sampling Intervals was reported for the Structure footprint (SF) (Table 5.2). During the first sampling interval (Figure 5.2 a and c), farmland RCA3 presented a significantly lower SF compared to the two reference sites (undisturbed NVR and RConv farmland), indicating that this farmland has reduced activity of nematodes from higher trophic levels compared to those recorded for the reference sites. The lack of significant differences among the CA farmlands further indicated similarity in terms of the activity and roles of such nematodes in these farmlands. Farmland RCA2 presented the highest SF of the three CA farmlands not differing significantly from the undisturbed NVR reference site or the disturbed reference site RConv farmland, while farmlands RCA1 and RCA3 presented significant differences from NVR. However, during the second sampling interval (Figure 5.2 b and d), the CA farmlands generally presented increased SFs, while the SFs of both reference sites decreased. Farmland RCA1 furthermore presented a significantly higher SF than that of the reference site RConv farmland.

Significant differences between treatments and intervals were reported for the Herbivore footprint (HF) along with a significant interaction for Treatments*Sampling intervals (Table 5.2) indicating that the activity of herbivorous nematodes was influenced by specific factors in the soil ecosystem over time. During the first sampling interval, farmland RCA3 presented the highest HF differing significantly from those of RCA1 and RConv (Table 5.2). During the second sampling interval, all treatments, however, presented increased Herbivore footprints with RConv having the highest HF (Figure 5.2 b and d). The HF of the latter farmland furthermore differed significantly from all the other treatments.

Significant differences were reported between the treatments in terms of the Predator footprint (PF), while no significant interaction for Treatments*Sampling Intervals were reported (Table 5.2). Farmland RCA3 presented a PF of zero during the first sampling interval (Table 5.2; Figure 5.2 a and d) and only differed significantly from NVR that had the highest PF, while the other treatments did not differ significantly from NVR. The same trend was evident during the second sampling interval (Figure 5.2 b and d), with RCA3, however, presenting an increased PF.

A significant interaction was reported for Treatments*Sampling Intervals in terms of the Omnivore footprint (OF) (Table 5.2) indicating that clear changes occurred in the activity of omnivorous nematodes over time. Farmlands RCA1 and RCA3 was the only treatments that presented a significantly lower omnivore activity compared to the undisturbed NVR reference site, which had the highest OF during the first sampling interval (Figure 5.2 a and d). The lowest OF was presented by RCA3 that also displayed a significant difference from the reference site RConv, but not from the other CA farmlands. During the second sampling interval (Figure 5.2 b and d), the only significant difference was between RCA1 that presented the highest OF and RConv with the lowest. The CA farmlands all presented increased OFs, especially RCA1 that presented the largest. The two reference sites both presented decreased Omnivore footprints indicating reduced omnivore activity over time.

Significant differences were reported between treatments in terms of the Fungivore footprint (FF) and no significant interaction was reported for Treatments*Sampling Intervals. Farmland RCA1 presented the highest FF and differed significantly from RCA2 and RConv with lower FFs. No significant differences were furthermore reported among RCA1, RCA3, and NVR. A similar trend was reported during the second sampling interval where RCA1 again presented the highest FF indicating similar conditions in the soil food web than during the first sampling interval (Table 5.2; Figure 5.2 b and d).

No significant differences were reported between the treatments or sampling intervals and no significant interaction was reported for Treatments*Sampling Intervals for the Bacterivore footprint (BF) (Table 5.2). This indicated that there were no distinct differences among the soil food web conditions of the different treatments and that no changes occurred over the sampling intervals in terms of the activity of bacterivores (Figure 5.2 b and d). The focus will therefore be on the Structure, Herbivore, Predator, Omnivore, and Fungivore footprints since these metabolic footprints yielded significant differences.

It was reported by Da Silva *et al.* (2021) that the SF presented variability based on different management practices as well as seasonal changes (e.g., precipitation). Similarly, in this study, it may be possible that these factors impacted the SF over time. The lack of significant differences between the CA farmlands in both sampling intervals (Table 5.2) might therefore also be due to the similar management practices and crop rotations of the farmlands. Farmland RCA3, which presented the lowest SF during the first sampling interval (Figure 5.2 a and c), however, indicated reduced soil ecosystem functioning due to the lack of activity of nematodes belonging to higher trophic levels. During the first sampling interval, the soil food web of RCA2 favoured sensitive nematodes having a regulatory function (Ferris *et al.*, 2001; Ferris *et al.*, 2012a).

Table 5.2: Mean ± standard error of nematode metabolic footprint values (Enrichment, Structure, Herbivore, Predator, Omnivore, Fungivore, and Bacterivore footprints) of three conservation agriculture farmlands (RCA1-3), a conventional agriculture farmland (RConv), and a natural veld area/reference site (NVR) in Reitz. Based on factorial analysis common superscript indicates no significant differences (p<0.05) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling interval					**Second Sampling interval**							
Index	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**Effect**	**F ratio**	**p-value**
Enrichment footprint	^{a}34,43± 7,25	^{a}14,73± 2,18	^{a}34,81± 9,87	^{a}28,87± 11,39	^{a}73,40± 57,54	^{a}30,06± 7,01	^{a}19,86± 5,47	^{a}22,92± 8,94	^{a}10,92± 3,37	^{a}20,43± 4,98	Treatment Interval Treatment* Interval	1,45 2,35 0,50	0,230 0,131 0,735
Structure footprint	^{ab}29,59± 8,85	^{abc}84,78± 31,60	^{a}8,34± 5,68	^{bc}137,94± 37,21	^{c}170,70± 28,13	^{c}166,43± 40,08	^{abc}118,55± 31,00	^{abc}57,90± 19,21	^{ab}33,40± 13,82	^{abc}109,17± 20,05	Treatment Interval Treatment* Interval	4,35 0,43 6,69	0,004 0,515 0,000
Herbivore footprint	^{a}93,47± 20,18	^{ab}184,86± 44,19	^{bc}395,92± 67,96	^{a}157,23± 57,24	^{ab}136,96± 19,06	^{ab}222,12± 44,60	^{bc}395,59± 87,88	^{bc}736,22± 59,97	^{d}1239,36± 271,56	^{ab}170,41± 20,52	Treatment Interval Treatment* Interval	12,78 46,30 6,07	0,000 0,000 0,000
Predator footprint	^{ab}17,41± 8,25	^{ab}25,53± 8,02	^{a}0	^{ab}23,02± 10,34	^{b}29,32± 6,29	^{ab}41,87± 12,82	^{ab}17,09± 3,57	^{a}16,97± 5,83	^{ab}16,58± 10,51	^{b}54,41± 13,15	Treatment Interval Treatment* Interval	3,98 3,45 1, 030	0,007 0,069 0,150
Omnivore footprint	^{ab}9,83± 4,35	^{abcd}48,23± 22,00	^{a}4,49± 4,49	^{bcd}111,45± 33,38	^{d}127,89± 27,99	^{cd}115,32± 31,36	^{abcd}86,46± 31,04	^{abcd}33,48± 18,03	^{abc}13,77± 11,83	^{abcd}42,39± 11,35	Treatment Interval Treatment* Interval	2,40 0,02 7,62	0,063 0,882 0,000
Fungivore footprint	^{b}29,31± 5,56	^{a}12,03± 2,76	^{ab}26,24± 7,84	^{a}15,02± 4,73	^{ab}25,92± 5,24	^{b}25,70± 2,99	^{a}12,61± 4,26	^{ab}10,76± 2,89	^{a}13,97± 3,22	^{ab}21,08± 3,75	Treatment Interval Treatment* Interval	3,75 2,84 0,94	0,010 0,098 0,446
Bacterivore footprint	^{a}60,55± 14,59	^{a}55,93± 21,07	^{a}123,31± 26,11	^{a}66,10± 17,04	^{a}97,63± 67,56	^{a}97,42± 19,42	^{a}62,10± 13,25	^{a}77,74± 16,18	^{a}53,39± 8,45	^{a}46,14± 9,35	Treatment Interval Treatment* Interval	2,25 0,02 0,93	0,077 0,880 0,453

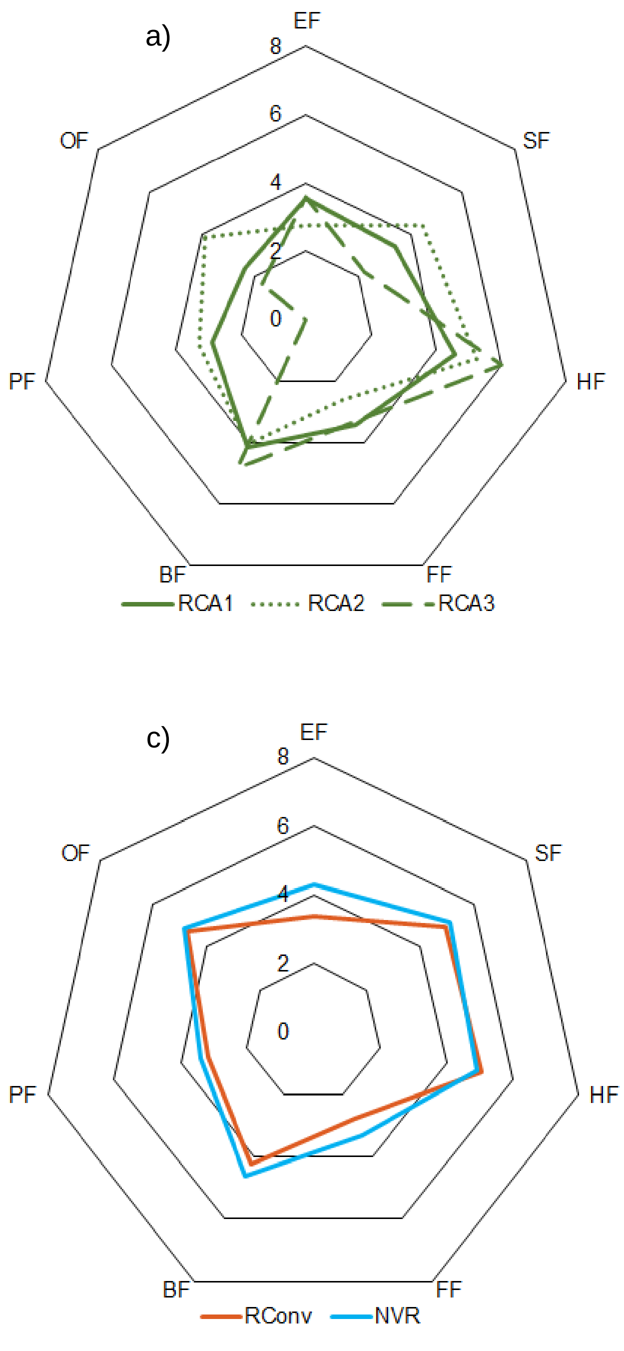


Figure 5.2: The nematode metabolic footprints (EF = Enrichment footprint; SF = Structure footprint; HF = Herbivore footprint; FF = Fungivore footprint; BF = bacterivore footprint; PF = Predator footprint; OF = Omnivore footprint) for three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv), and one natural veld area/reference site (NVR), in Reitz during the first (a, c), and second (b, d) sampling intervals. Values are the mean of six replicates; n = 6, and data are ln transformed (Lazcano *et al.*, 2021; Sánchez-Moreno *et al.*, 2018).

The reason for the high SF of RConv is, however, unclear, while the high SF of the undisturbed reference site NVR agrees with reports that nematodes belonging to higher trophic levels have been found to be more active and abundant in undisturbed areas (Maina *et al.*, 2020; Tenuta & Ferris, 2004).

During the second sampling interval (Figure 5.2 b and d), RCA1 displayed the highest SF, differing significantly from RConv that displayed a decreased SF from the first sampling interval along with NVR (Table 5.2). The increased SF of the CA farmlands during the second sampling interval might be, as discussed, resulting from increased precipitation and the beneficial impact that CA practices may have on functioning of the soil food web. The reason for the reduced SF of the undisturbed reference site NVR during the second sampling interval is, however, unclear, while the SF reduction in RConv indicated soil ecosystem disturbance possibly due to the adverse effect of conventional tillage (Sánchez-Moreno *et al.*, 2018) and fertilization (Kou *et al.*, 2020) associated with maize cultivation.

In agricultural farmlands, herbivores are impacted by root exudates and living plant tissue that is the primary suppliers of C to the soil food web (Ewald *et al.*, 2020; Glavatska *et al.*, 2017). The type of crop that is cultivated can furthermore impact the occurrence and activity of herbivorous nematodes (Ewald *et al.*, 2020; Yeates *et al.*, 1993). Differences between treatments and the significant interaction for Treatments*Sampling Intervals (Table 5.2) might therefore be explained by cash crop rotation that occurred between the two sampling intervals. Farmland RCA3 presented the highest HF of all the treatments during both sampling intervals (Figure 5.2 a-d) and even though it was under the same cash crop rotation as the other CA farmlands, it differed significantly from RCA1 with the lowest HF. The reason for this variation remains unclear. Farmland RCA3 further differed significantly from RConv, for which the low HF also cannot be explained at this stage. However, supporting the latter trend is that Lazarova *et al.* (2021) also found increased herbivory in farmlands under reduced tillage when compared to conventional tillage. Also, Bongiorno *et al.* (2019a) reported a 70 % increase in herbivore numbers under reduced tillage in comparison to conventional tillage. No significant difference was furthermore reported between RCA3 and NVR, which might be explained by the possibility of the increased occurrence of herbivores in undisturbed natural areas as a higher diversity of plant species occurs resulting in an increased host range for plant-parasitic nematodes (Girgan *et al.*, 2020; Hodda *et al.*, 2009).

The overall increase in the HFs in all the treatments may be due to the presence of roots in the soil during the growing season that will subsequently increase the metabolic activity of plant-parasitic nematodes (Ferris *et al.*, 2012a; Luo *et al.*, 2021). This can typically also occur due to increased precipitation as plant-parasitic nematodes are more active in a moist rhizosphere (Da Silva *et al.*, 2021). Farmland RConv, however, presented the highest HF during the second sampling interval (Table 5.2; Figure 5.2 b and d) and maize monocropping systems are generally characterized by the occurrence of higher plant-parasitic nematode numbers (Postma-Blaauw *et al.*, 2010; Zhang *et al.*, 2015b). Increased fertilization that stimulates plant growth and root development may therefore also result in increased HFs (Herren *et al.*, 2020; Maina *et al.*, 2020) as was the case for RConv.

The significant differences that occurred between the treatments in terms of the PF might be explained by the sensitivity of this group to disturbance (Bongers & Ferris, 1999; Shaw *et al.*, 2019). The exact reason for the Predator footprint of zero reported for RCA3, and its increase during the second sampling interval (Table 5.2; Figure 5.2 b and d), remains unclear, but disturbance to the soil ecosystem is known to result in reduced occurrence of predatory nematodes (Shaw *et al.*, 2019). Agreeing with literature is that the undisturbed reference site NVR presented the highest PF during both sampling intervals, confirming that its undisturbed soil food web has regulatory functions performed by higher cp-value nematodes (Liang *et al.*, 2020; Zhang *et al.*, 2013).

Environmental changes, amongst other factors, mainly influence omnivorous nematodes in the soil food web (Coutinho *et al.*, 2018; Yeates *et al.*, 1993), which explains the significant interaction that occurred between Treatments*Sampling Intervals for the OF. The OF increased in the CA farmlands during the second sampling interval (Figure 5.2 b and d) indicating that these treatments might have had more optimal pest suppression services active within their soil ecosystems due to an environment that benefitted the occurrence of omnivores (Ferris, 2010; Maina *et al.*, 2020). It might also have been contributed by to the derived benefits of CA to the soil ecosystem (Henneron *et al.*, 2014). Farmlands under crop rotation, like the CA farmlands, are furthermore known to favour the occurrence of high trophic level nematodes like omnivores (Zhong *et al.*, 2015). The reasons for the decreased OF at the reference site NVR are, however, unclear, but for RConv it might be explained by the addition of increased inorganic fertilization during the second sampling interval (Cheng *et al.*, 2021; Song *et al.*, 2016).

In terms of the FF, which is also subject to variability due to environmental disturbance, significant differences were reported between treatments (Table 5.2). This indicated, as with the SF and PF, that specific management practices led to varied disturbance that affected functioning in the soil food web. The lack of significant differences between RCA1, RCA3, and NVR indicate that no-tillage, which can cause an increase in fungivore activity (Zhang *et al.*, 2015b), and undisturbed areas may present similar degrees of soil ecosystem functioning in terms of mineralization services (Maina *et al.*, 2020). The reason for the low FF in RCA2, not differing significantly from reference farmland RConv is, however, unknown. But the low value in the latter reference site agrees with a report by Zhang *et al.* (2015b) that decreased fungivore activity occur under conventional tillage, which can most probably be ascribed to the addition of inorganic fertilizer (Wei *et al.*, 2012).

5.3 Other bioindicators of soil ecosystem health

The selected soil health bioindicators AC, SR, and % OM were used additionally to the NBIs to indicate progress in the restoration of soil ecosystem health and functioning in farmlands under CA (Nunes *et al.*, 2018; Williams *et al.*, 2020). The mean and standard error for the measured bioindicators is presented in Table 5.3.

A significant difference ($p<0.05$) between the treatments and a significant interaction was reported between Treatments*Sampling Intervals in terms of SR indicating changes in SR over time among the treatments. During the first sampling interval, RCA3 presented the highest rate of SR and differed significantly from all other farmlands. In contrast, RCA1 presented the lowest SR rate. However, during the second sampling interval, RCA3 presented decreased SR and did not differ significantly from RCA2 or NVR, but still presented the highest SR rate differing significantly from RCA1 and RConv. Farmland RCA1 again presented the lowest SR rate.

A significant difference was reported between the treatments in terms of the AC content while no significant difference occurred between the sampling intervals and no significant interaction between Treatments*Sampling intervals were reported. This indicated that no changes occurred among the treatments over time in terms of the AC content. During the first sampling interval, the AC content of all three CA farmlands differed significantly from each other. Farmlands RCA1, RCA2, and RConv also presented significant differences from NVR that had the highest AC content, while RCA3 differed significantly from RConv, but not from NVR. It was furthermore reported that RCA1 differed significantly from all the other treatments by presenting the lowest AC content. The same trend occurred during the second sampling interval.

In terms of the % OM, no significant differences were reported between the treatments or between sampling intervals with no significant interaction reported for Treatments*Sampling Intervals. This might possibly be because OM levels in the soil respond much slower than indicators like SR or AC (Saini *et al.*, 2021).

Bioindicators like SR and AC are known to be sensitive (Saini *et al.*, 2021; Seifu & Elias, 2018); especially SR, that can be affected by various abiotic soil properties, seasonal variation, and changes in land management (Du *et al.*, 2020; Ebrahimi *et al.*, 2019). This possibly explains the significant interaction that occurred for SR. Even though the CA farmlands were under the same crop rotation, variations in terms of the bioindicators were still observed among the three CA farmlands. It was furthermore found by Williams *et al.* (2020) that bioindicators like SR and AC are strongly correlated with soil texture, while increased SR was reported for soils with a high clay % in a study by Van Diepeningen *et al.* (2006). Of all the treatments, farmland RCA3 presented the highest rate of SR during both sampling intervals, while also having the highest clay %. This while RCA1 presented the lowest SR rate also having the lowest clay %. Similar results were also reported for % OM and partially for AC. These trends are, however, further investigated in Section 5.4 through the use of RDAs.

The undisturbed reference site NVR and disturbed reference site RConv farmlands presented no significant differences in SR during both sampling intervals. Possible reasons for this remain unclear. The reference site NVR however presented increased SR during the second sampling interval while only differing significantly from RCA1 that had the lowest SR. The reason for this increase is unclear but may be ascribed to seasonal variation as the second sampling interval occurred during the growing season with SR being sensitive to seasonal variation (Du *et al.*, 2020). It was the undisturbed reference site NVR that presented the highest AC content of all treatments during both sampling intervals. This is an expected trend for undisturbed environments as opposed to environments under agricultural management that may rather present lower AC measures (Ghosh *et al.*, 2019a). The AC content of the reference site NVR did furthermore not differ significantly from that of RCA3 for which the reason remains unclear at this stage. No significant differences were reported among the treatments for the % OM. The reference site NVR, that was not subject to high levels of soil disturbance did, however, not present the highest % OM of all treatments as would be expected of such an environment (Sprunger *et al.*, 2019). Reasons for the observed variation in the bioindicators, especially among the CA farmlands are further elaborated in Section 5.4.

Table 5.3: Mean ± standard error of the measured bioindicators (soil respiration - SR, active carbon - AC, and total percentage organic matter - % OM) of three conservation agriculture farmlands (RCA1-3), a conventional agriculture farmland (RConv), and a natural veld area/reference site (NVR) in Reitz. Based on factorial analysis common superscript indicates no significant differences ($p<0.05$) between treatments per sampling interval. The F ratios and p-values for treatments, sampling intervals, and interaction (Treatment*Interval) are also provided, and significant values indicated in red.

	First Sampling interval					**Second Sampling interval**							
Bioindicator	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**Effect**	**F ratio**	**p-value**
SR (ppm)	^{a}16,96± 1,70	^{abc}32,32± 4,48	^{d}77,73± 13,34	^{ab}25,68± 4,67	^{abc}37,53± 5,46	^{a}19,74± 0,80	^{abc}31,01± 3,99	^{cd}54,18± 5,21	^{ab}25,46± 2,34	^{bc}51,31± 4,00	Treatment Interval Treatment* Interval	22,02 0,23 2,91	0,000 0,635 0,031
AC (mg/kg)	^{c}52,63± 15,56	^{a}176,25± 14,91	^{b}262,52± 25,37	^{a}125,58± 19,55	^{b}274,39± 20,89	^{c}49,01± 12,83	^{a}166,99± 8,25	^{b}193,12± 19,41	^{a}132,02± 18,20	^{b}257,03± 16,30	Treatment Interval Treatment* Interval	45,33 2,77 1,40	0,000 0,102 0,246
% OM	^{a}0,80± 0,06	^{a}2,00± 0,14	^{a}3,10± 0,29	^{a}2,60± 0,12	^{a}2,45± 0,11	^{a}0,92± 0,10	^{a}2,17± 0,21	^{a}3,08± 0,25	^{a}2,85± 0,20	^{a}2,63± 0,15	Treatment Interval Treatment* Interval	1,89 1,84 0,91	0,128 0,181 0,466

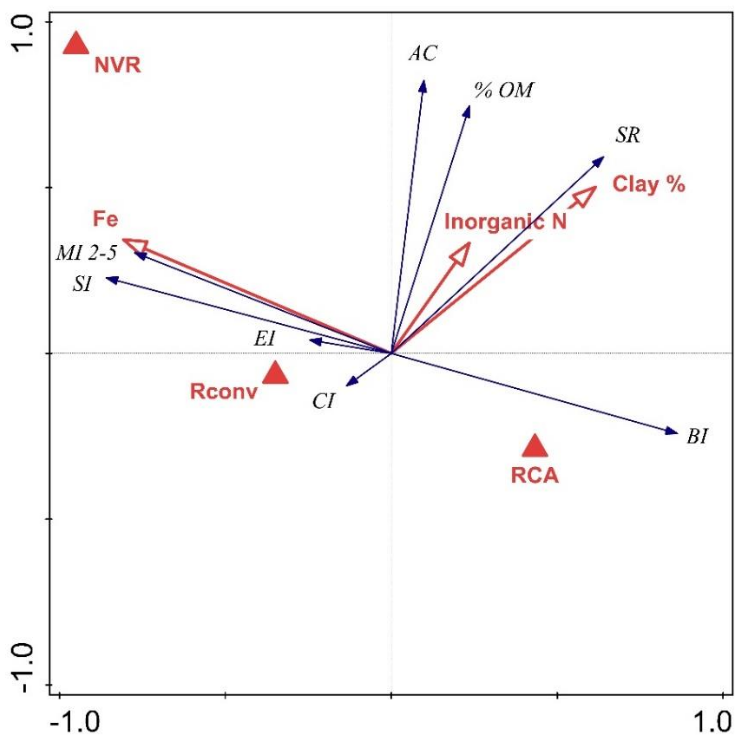
5.4 The effect of abiotic soil factors on soil ecosystem health and functioning

Key abiotic properties that potentially influenced the soil ecosystems of the studied farmlands were investigated using redundancy analyses (RDAs). In the latter analyses, the abiotic parameters were included as explanatory variables and soil ecosystems measurements as response variables. The values of measured abiotic properties are listed in Table S5.2. Soil ecosystem measurements are represented by NBIs, AC, SR, and % OM (Table 5.1 and 5.3). The results for the two sampling intervals are illustrated on biplots (Figure 5.3 a and b).

The RDA for the first sampling interval (Figure 5.3 a), with 55.38 % variation explained on axis 1 (32.88 %, $p<0.05$) and axis 2 (22.50 %, $p<0.05$), indicated that soil ecosystem measurements were impacted by the selected soil abiotic properties. The CA farmlands presented a positive correlation with the BI and a negative correlation with the CI, EI, MI 2-5, SI, and iron (Fe) during the first sampling interval. The reference site RConv was positively correlated with the CI, EI, MI 2-5, SI and Fe, while NVR presented a positive correlation with the SI, MI 2-5, and Fe. A positive correlation further existed between the clay % and the bioindicators AC, SR, and % OM, which is also positively correlated with inorganic N. In this RDA, the treatments were responsible for 21.6 % ($p<0.05$) of the observed variation in response variables, Fe for 24.8 % ($p<0.05$), clay % for 15.1 % ($p<0.05$), and inorganic N for 6.6 % ($p<0.05$).

The RDA for the second sampling interval (Figure 5.3 b), with 35.11 % variation explained on axis 1 (26.22 %, $p<0.05$) and axis 2 (8.89 %, $p<0.05$), showed that there was a significant impact of the soil abiotic properties on soil ecosystem processes. The CA farmlands presented a positive correlation with the SI and MI 2-5. The reference site RConv was positively correlated with the BI and CI, while NVR presented a positive correlation with the EI, AC, SR, % OM, and clay %. A high clay % was furthermore positively correlated with the bioindicators, AC, SR, and % OM. In this RDA the treatments were responsible for explaining 30.4 % ($p<0.05$) of variation and clay % 14 % ($p<0.05$).

a)



b)

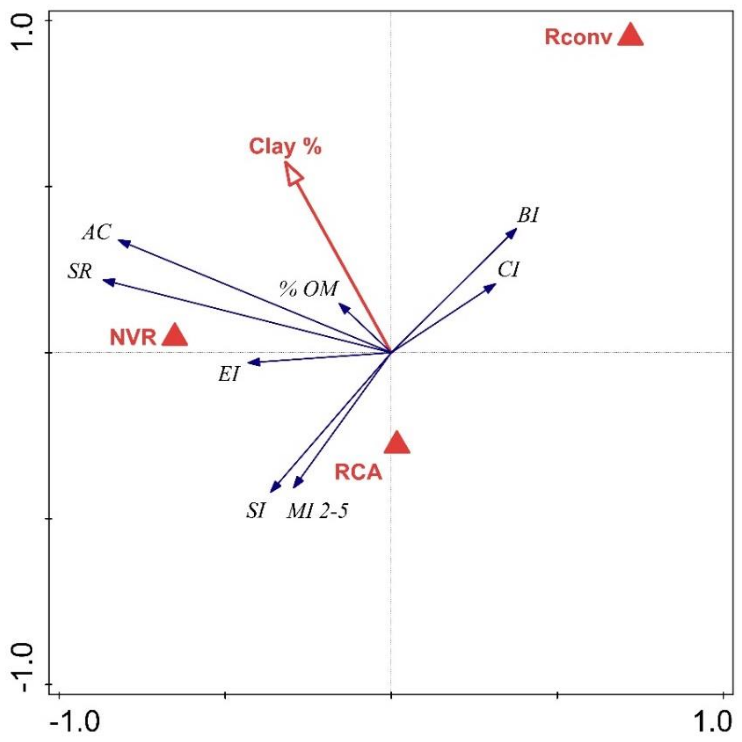


Figure 5.3: Redundancy analysis indicating the correlation among soil abiotic parameters (explanatory variables) and bioindicators (response variables) of soil ecosystem health and functioning for the first (a) and second (b) sampling intervals in Reitz (RCA = Reitz conservation agriculture farmlands, RConv = Reitz conventional agriculture farmland, NVR = Reitz Natural veld area/reference site, MI 2-5 = Maturity index 2-5, EI = Enrichment index, SI = Structure index, BI = Basal index, CI = Channel index, SR = Soil respiration, AC = Active carbon, % OM = Percentage total organic carbon).

5.4.1 *The positive correlation between clay % and bioindicators*

During the first sampling interval, clay % was positively correlated with the bioindicators AC, SR, and AC (Figure 5.3 a), it was also positively correlated with these bioindicators and the EI during the second sampling interval (Figure 5.3 b). Similar results have been reported in studies by Idowu *et al.* (2009) and Nunes *et al.* (2018) where increased levels of AC, SR, and % OM were observed for soils with an increased clay content (i.e., finer-textured soils). It may furthermore be possible that the increased clay % have led to increases in enrichment nematode abundance (e.g., bacterivores) as observed by Yeates (2003). The latter author suggested that the porosity of soils with a high clay % potentially favoured the occurrence of bacterivorous nematodes.

Soil ecosystem health and functioning are largely impacted by soil texture and variations in soil texture are therefore important to consider when assessing soil ecosystem functioning (Moebius-Clune *et al.*, 2016). A possible reason for the correlation between AC and clay % is that the clay content of soils plays an important role in the C storing capacity of soils (Li *et al.*, 2018; Six *et al.*, 2004). There are furthermore many factors that actively impacts SR. An example include increased clay % in soils that may induce increased SR as a result of the soil's increased capacity to retain soil moisture to be available to soil fauna that contributes to SR (Ebrahimi *et al.*, 2019). The % OM in soils are also known to increase with high clay % (Oades, 1988; Williams *et al.*, 2020). The bioindicators AC, SR, and % OM are furthermore closely related possibly explaining similarity in their response to an increased clay content (Williams *et al.*, 2020).

The clay % was further positively correlated with inorganic N that also presented a positive correlation with the bioindicators. This was, however, not a strong positive correlation, but inorganic N has been found to increase measures of the bioindicators AC, SR, and % OM through its immediate supply of organic and inorganic C and N that are utilizable by soil biota (Lazcano *et al.*, 2021). This can, however, only occur to a certain degree whereafter inorganic N starts to adversely affect the soil ecosystem (Chen *et al.*, 2018).

5.4.2 *The role of Fe in the soil ecosystem and its correlation with the reference sites*

During the first sampling interval, Fe was positively correlated with the MI 2-5, EI, and SI (Figure 5.3 a). It was furthermore associated with the undisturbed (NVR) and disturbed (RConv) reference sites. The presence of Fe in soils was found by Bai *et al.* (2011) to be mainly sourced from natural soil processes like the weathering of parent material (Al-Wabel *et al.*, 2017). The continued cultivation of maize was also found by Strom *et al.* (2020) to result in increased Fe levels in soils. The mobility of Fe is, however, determined by soil pH and a soil pH ≥ 7 is expected to render Fe unavailable to plants, while acidification may increase its solubility (Filipek-Mazur *et al.*, 2019;

Krohling *et al.*, 2016). The positive correlation between Fe and the EI might indicate that Fe may be sourced from the addition of inorganic fertilizer as Fe are a common constituent of fertilizers (Filipek-Mazur *et al.*, 2019) and Rconv received liberal amounts of inorganic fertilizer.

5.5 Conclusion

The variation in terms of the NBIs recorded between the CA farmlands is an interesting outcome for this study area, which cannot be explained. In general, however, healthier soil ecosystems and increased soil ecosystem functioning were reported for the CA farmlands during the second sampling interval. The healthier soil ecosystems of RConv (during the first sampling interval) could also not be explained, but it is suggested that the cultivation of soybean might have benefitted the soil ecosystem to some extent.

The studied CA farmlands and reference sites all exhibited varied soil textures that especially affected the bioindicators AC, SR, and % OM, as was evident from the RDAs. The farmlands with the highest reported clay % were positively correlated with AC, SR, and % OM. Similarly, in a study by Williams *et al.* (2020), it was reported that finer-textured soils presented increased AC, SR, and % OM. Soil physical parameters like soil texture must therefore be considered by land managers when making management decisions to increase crop production and agricultural sustainability.

During the first sampling interval of this study, inorganic N was also positively correlated with the selected soil health bioindicators. This was, however, not a strong correlation and the adverse effects of inorganic N on soil ecosystem health cannot be ignored. It furthermore seems that Fe increased soil ecosystem health by positively correlating to the MI 2-5 and SI. Possible reasons for this trend are, however, also unclear and further investigations are suggested. It is, however, suggested that Fe has been sourced from inorganic fertilizer application especially associated with RConv.

CHAPTER 6: CONCLUDING CHAPTER

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6.1 Testing of hypotheses

This study was firstly aimed at assessing the restoration of soil ecosystem health and functioning under conservation agriculture (CA) and its associated good agriculture practices. The latter included cover cropping and cash crop rotation in Vrede (farmlands with a uniform soil texture) and cash crop rotation in Reitz (farmlands with variation in soil texture). Knowledge generated from previous projects on the soil texture differences at Reitz prompted the second aim of this study, namely identifying key soil abiotic properties that impact the restoration and functioning of the studied soil ecosystems. Soil abiotic properties may have major effects on soil biological processes and must be included in soil ecosystem studies (Van Eekeren *et al.*, 2010; Vincent *et al.*, 2018). Results of the two study localities were therefore not directly compared. By means of completing the objectives, the aims of this study were achieved, and the hypotheses were proven. As a summary, the hypotheses are stated and discussed below:

1. The soil ecosystems of farmlands under CA will present increased recovery and functioning after transitioning from Conv practices.

The implementation of CA has been shown to enhance soil ecosystem health and functioning (Alikhani *et al.*, 2018; Kassam *et al.*, 2019). Similarly, results from this study evidenced the restoration of soil ecosystem health and functioning at the CA farmlands in Vrede. This was measured against the undisturbed reference site NVV, which presented the overall highest measures of soil ecosystem health, as well as the disturbed reference site VConv that displayed decreased soil ecosystem health. For the majority of the NBIs, the CA farmlands did not differ significantly from NVV, while displaying significant differences from VConv, indicating a definite degree of soil ecosystem restoration in the CA farmlands (Jat *et al.*, 2012; Kassam *et al.*, 2019). The CA farmland VCA2, however, presenting decreased soil ecosystem health and function compared to the other CA farmlands. This farmland also presented the largest Bacterivore (BF) and Enrichment footprint (EF), which could be the result of inorganic N fertilization (Lazcano *et al.*, 2021; Maina *et al.*, 2020). However, it is important to consider that this farmland was in a different crop rotation sequence that the other CA farmlands (VCA1 and VCA3).

In terms of the selected bioindicators, NVV again presented the highest measures of active carbon (AC) content, soil respiration (SR), and percentage total organic matter (% OM). This confirms the expected higher degree of soil ecosystem health of an undisturbed environment

(Sprunger *et al.*, 2019). Even though there were no significant differences between the CA farmlands and VConv for AC and SR, two of the three CA farmlands had a higher % OM than VConv. The low % OM is expected of a farmland under Conv since OM is rapidly decomposed in Conv systems due to intensive tillage (Sanaullah *et al.*, 2020; Sithole *et al.*, 2016). The implementation of CA is, however, known to increase the OM content of soils (Sarkar *et al.*, 2020). This trend was observed for most of the CA farmlands emphasizing progression toward soil ecosystem recovery as an increased % OM in agricultural farmlands enhances soil ecosystem health and functioning (Adetunji *et al.*, 2020; Williams *et al.*, 2020).

In Reitz the CA farmlands presented variation in soil ecosystem health and functioning; displaying reduced measures of NBIs, especially during the first sampling interval prior to the growing season. Improvements were, however, observed during the second sampling interval through increased values for the Maturity index 2-5 (MI 2-5) and the Structure index (SI). The reason for this improvement is, however, unclear. For this reason, the duration of a follow-up study concerning this trend must be over multiple years and specifically aimed at the possible effects of the growing season and its temporal effects on soil ecosystem functioning. Important to note is that reasons for the increased soil ecosystem functioning presented by farmland RCA2, above the other CA farmlands, remains unknown since the possible parameter that led to its improved soil ecosystem health and functioning might have not been measured in this study.

The prominent Herbivore footprints (HF) observed in the CA farmlands and the disturbed reference site RConv indicate that definite activity of herbivorous nematodes took place, especially during the second sampling interval. The largest HF for RConv agrees with findings of increased activity of herbivorous nematodes in Conv systems especially under maize (*Zea mays*) cultivation (Postma-Blaauw *et al.*, 2010; Zhang *et al.*, 2015b). In terms of the bioindicators, variation was observed among the CA farmlands despite the similarity in the applied management practices. This variation was further investigated in redundancy analyses (RDAs) that formed part of the second hypothesis.

The first hypothesis is therefore accepted in the study on the Vrede CA farmlands. The CA farmlands of Reitz presented varying results, which remain inconclusive.

2. Soil chemical and physical properties, which represent the abiotic component of soil health, influences biological processes and therefore soil ecosystem functioning.

According to the RDAs, inorganic nitrogen (N) correlated with the Basal index (BI) for the Vrede study and presented a negative correlation to the remaining NBIs and the selected bioindicators. This confirmed the adverse effect of inorganic fertilizer application on soil ecosystem health and functioning (Lazcano *et al.*, 2021; Wei *et al.*, 2012). Soil pH also positively impacted measures of the bioindicators and benefitted the soil ecosystem though increases in the MI 2-5, SI, Channel index (CI), and Enrichment index (EI). The CA farmlands was further associated with an increased soil pH highlighting the benefits of CA to the soil ecosystem as it may buffer soil acidification (Duiker & Beegle, 2006). However, the presence of heavy metals Copper (Cu), Nickel (Ni), and Cobalt (Co) in the CA farmlands adversely impacted soil ecosystem health by being negatively correlated with the bioindicators. The EI was further positively correlated with Cu and Ni indicating that these metals might have been sourced from inorganic fertilizer application (Naccarato *et al.*, 2020).

In Reitz it was found that an increased clay % benefitted the soil ecosystem through its positive correlation with the measured bioindicators. Similar results were reported by Nunes *et al.* (2018) and Williams *et al.* (2020). It is therefore evident that differences in soil texture strongly impacted soil ecosystem health. Interestingly, the positive correlation of Iron (Fe) with the SI, EI, and MI 2-5 could not be confirmed by literature. Although these results indicated increased soil ecosystem health with increased Fe content, a lack of information exists on the impact of Fe on soil ecosystem health. Its positive correlation with the EI might, however, indicate that increase Fe levels in the soil ecosystem may be sourced from inorganic fertilizer application.

The second hypothesis is accepted in both study localities where abiotic soil properties impacted soil ecosystem health and functioning.

6.2 Future recommendations

The benefit of CA implementation was highlighted in this study as it was shown to restore soil ecosystem health and functioning. This was especially evident in farmlands with a uniform soil texture, such as for the Vrede study site. Variation in soil texture was demonstrated to have an important impact on soil ecosystem health and functioning at the Reitz study site and presents an opportunity for further investigations.

The impact of abiotic soil properties on soil ecosystem health are evident from literature and results emanating from this study, but agricultural management practices can, however, impact such abiotic soil properties (Sanaullah *et al.*, 2020; Sithole *et al.*, 2016). This must be considered by farmers as their chosen agricultural practices may therefore indirectly manage belowground interactions. Examples of this includes the beneficial impact of increased soil pH on soil ecosystem health. Farmers must therefore realise the importance of implementing practices that reduce and buffer soil acidification. The adverse effect of inorganic N on the soil ecosystem was also highlighted as a result of this study, and a reduction in its application is important for active soil ecosystem recovery. Furthermore, increased use of synthetic agrochemicals is a concern in CA as pests and weeds are not primarily managed through tillage (Sanaullah *et al.*, 2020). A build-up of heavy metals sourced from such chemicals might pose a threat to soil ecosystem health as was displayed in this study.

Reduced inorganic fertilizer application is already implemented by CA farmers, but there is a further need for reduced pesticide use in CA systems. Therefore, further agronomical studies must be aimed at practical means to reduce its use in CA systems without compromising yield. Despite management practices, abiotic factors like clay % may impact soil ecosystem health and functioning to a larger degree. This must therefore be considered by farmers when management decisions are made, e.g., implementing more than one good agricultural practice on farmlands with a lower clay % to compensate for possible adverse effects imposed by e.g., a higher sand % to therefore still aid soil ecosystem restoration. Soil texture played an important role in this study, but not all abiotic soil properties were measured due to logistical reasons. It is therefore suggested that parameters like soil moisture, water holding capacity, and aggregate stability are included in future studies to identify possible means to indirectly manage soil ecosystem health and functioning.

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SUPPLEMENTARY MATERIAL

Table S4.1a: Mean ± standard error of the mean for the abundance of nematode families and genera (per 200 g of soil) found in three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede during two sampling intervals. Family and genera classification under respective trophic groups are based on the Nemaplex classification.

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Bacterivores										
Acrobeles	0	2,13± 2,13	16,47± 14,46	83,40± 45,62	32,98± 8,77	1,37± 1,37	1,28± 1,28	0	31,25± 18,46	36,18± 14,71
Alaimidae	0	2,95± 2,95	0	16,56± 7,77	5,07± 2,40	0	6,29± 3,28	1,41± 1,41	3,24± 2,08	5,88± 3,73
Alaimus	0	0	6,20± 3,49	0	1,21± 1,21	0	0	0	5,22± 3,30	0
Anaplectus	38,50± 12,18	60,20± 20,40	14,15± 6,97	102,92± 31,60	17,03± 6,21	28,25± 9,82	15,18± 3,25	11,16± 3,79	4,26± 2,87	10,39± 3,02
Cephalobidae	5,80± 5,80	7,77± 4,10	0	0	0	0	2,60± 2,60	0	0	0
Cephalobus	140,43± 25,36	115,70± 18,25	124,24± 37,00	267,12± 28,21	22,72± 6,14	39,06± 10,70	43,05± 18,88	47,59± 12,76	96,94± 14,36	18,73± 5,04
Cervidellus	6,47± 2,93	0	5,51± 5,51	14,00± 9,11	2,83± 1,80	10,05± 4,71	3,00± 1,92	6,72± 2,58	1,39± 1,39	0
Chiloplacus	0	0	0	0	3,80± 2,47	0	0	0	0	0
Cruznema	2,06± 2,06	0,93± 0,93	0	0	0	0	0	0	0	0
Diplogastridae	0	0	0	0	0	0	0	0	0	1,73± 1,73
Eucephalobus	51,84± 15,69	103,19± 32,06	32,38± 5,83	130,83± 38,78	15,82± 7,34	69,05± 50,88	61,09± 17,51	37,05± 11,06	75,28± 10,69	13,84± 2,41
Mesorhabditis	2,08± 2,08	10,29± 8,04	26,29± 14,94	30,69± 10,34	1,46± 1,46	16,33± 8,62	21,59± 9,77	8,31± 6,95	11,96± 2,92	3,84± 3,84

Table S4.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Monhysteridae	22,70± 8,08	10,32± 6,70	3,81± 3,81	45,53± 20,53	11,05± 3,82	5,41± 2,62	1,12± 1,12	12,16± 7,04	1,39± 1,39	8,86± 6,88
Panagrolaimus	8,52± 4,20	2,05± 2,05	30,37± 19,28	32,23± 10,07	11,62± 4,58	6,55± 3,19	18,86± 9,87	5,36± 1,71	10,02± 3,29	8,63± 5,78
Plectidae	0	0	0	0	0	0	0	0	0	3,13± 2,03
Plectus	54,36± 18,20	25,75± 7,84	77,20± 29,89	53,33± 24,23	23,11± 3,04	34,62± 12,38	10,54± 3,33	40,54± 8,44	32,83± 11,55	2,28± 1,50
Prismatolaimus	30,37± 6,98	6,23± 2,79	12,51±5,73	37,29± 16,34	9,13± 5,78	12,77± 6,11	2,25± 1,45	13,58± 3,84	24,43± 9,25	3,90± 1,86
Rhabditidae	0	0	1,88± 1,88	8,41± 8,41	0	2,55± 2,55	2,42± 1,54	0	1,27± 1,27	3,08± 3,08
Rhabditis	22,22± 8,22	207,68± 43,90	16,46± 10,02	40,52± 17,27	2,81± 1,79	7,50± 2,78	28,91± 10,20	8,00± 4,16	2,78± 2,78	1,54± 1,54
Teratocephalidae	0	0	0	0	1,46± 1,46	0	0	0	0	2,94± 1,86
Tylocephalus	4,92± 4,92	2,08± 2,08	17,49± 10,12	2,76± 2,76	14,27± 5,76	3,61± 2,56	0,95± 0,95	2,73± 1,73	3,24± 2,08	6,08± 2,92
Wilsonema	0	0	0	0	0	0	0	0	0	1,85± 1,85
Zeldia	0	10,47± 5,07	0	16,91± 7,51	3,00± 1,90	1,06± 1,06	3,31± 2,18	2,64± 1,67	18,50± 7,30	8,70± 6,81
Eucaryote feeders										
Achromadora	0	0	0	0	0	1,31± 1,31	4,00± 2,18	4,65± 2,74	0	4,98± 3,66
Fungivores										
Aphelenchoides	28,33± 19,38	2,13± 2,13	3,72± 2,35	59,44± 25,64	8,12± 2,82	25,87± 13,83	13,83± 6,81	12,23± 5,26	13,54± 3,31	37,09± 7,44

Table S4.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Aphelenchus	94,75± 18,11	106,53± 21,64	117,89± 25,60	226,47± 51,68	31,59± 12,80	85,81± 21,39	101,80± 21,12	111,27± 14,13	145,24± 27,10	20,62± 7,67
Diphtherophora	0	0	16,62± 12,66	0	0	2,55± 2,55	0	0	0	7,43± 4,90
Dorylaimoides	0	0	2,92± 1,87	0	0	0	0	0	0	0
Filenchus	0	0	16,19± 10,30	0	24,09± 9,48	52,23± 13,35	40,31± 11,11	17,31± 6,91	58,78± 11,23	111,83± 29,95
Leptonchidae	9,44± 7,26	0	0	0	8,73± 4,05	1,48± 1,48	0,95± 0,95	0	0	10,75± 7,28
Nothotylenchus	0	0	3,49± 2,21	0	0	5,37± 3,40	0,95± 0,95	0	0	5,38± 3,64
Tylencholaimellus	0	0	0	0	0	0	0	0	0	1,73± 1,73
Tylencholaimus	23,20± 10,13	2,95± 2,95	10,92± 3,48	0	34,54± 15,12	9,34± 6,47	0,95± 0,95	9,79± 6,72	1,62± 1,62	42,15± 23,26
Neotylenchidae	0	0,93± 0,93	0	3,99± 3,99	2,58± 2,58	4,69± 2,23	10,17± 3,28	0	1,39± 1,39	5,69± 2,71
Herbivores										
Anguina	0	0	0	0	0	0	0,95± 0,95	0	0	0
Axonchium	0	0	0,83± 0,83	0	2,50± 1,58	4,98± 2,31	8,74± 4,50	3,42± 2,77	4,62± 3,25	4,82± 3,22
Belondira	16,00± 5,94	5,96± 4,15	21,25± 10,12	8,65± 8,65	33,35± 13,29	13,32± 6,28	12,68± 3,87	11,61± 3,17	0	55,34± 22,58
Belondiridae	6,33± 4,15	2,05± 2,05	5,63± 5,63	0	8,68± 2,89	4,67± 3,23	6,10± 2,91	3,89± 2,68	1,62± 1,62	11,81± 10,22
Trichodorus	0	0	2,54± 2,54	0	0	0	0	0	0	0

Table S4.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Criconematidae	0	0	5,51± 5,51	5,52± 5,52	57,66± 27,81	0	0	0	0	0
Dolichodoridae	0	0	0	0	0	56,99± 21,63	0	17,00± 15,37	5,54± 2,72	27,86± 7,53
Ecphyadophoroides	0	0	5,51± 5,51	0	8,64± 4,52	0	0	0	0	6,91± 4,40
Helicotylenchus	0	0	0	0	0	16,38± 8,28	31,44± 12,31	5,44± 4,17	51,31± 19,24	85,55± 21,20
Hemicycliophora	0	0	0	0	0	0	0	0	0	2,80± 2,80
Hoplolaimidae	0	0	0	0	0	7,65± 7,65	2,13± 1,39	7,03± 2,02	4,16± 2,79	4,22± 1,90
Longidoridae	4,39± 2,81	6,15± 6,15	0	0	1,54± 1,54	2,55± 1,64	0	4,45± 2,56	0	3,70± 3,70
Meloidogyne	0	0	3,11± 2,01	0	0	0	0	0	0	0
Neopsilenchus	0	0	0	0	0	0	1,80± 1,14	0	0	0
Pratylenchus	212,09± 51,85	112,57± 16,14	129,50± 30,94	154,25± 44,83	9,18± 2,86	158,45± 29,77	91,01± 22,40	80,95± 8,47	94,31± 21,86	2,82± 1,80
Psilenchus	0	0	0	0	0	0	0	1,26± 1,26	0	0
Rotylenchulus	24,83± 10,76	28,92± 13,06	12,96± 6,91	50,06± 26,60	32,80± 11,99	22,75± 14,86	22,48± 10,89	21,25± 8,07	109,54± 59,58	3,84± 3,84
Scutellonema	75,67± 21,42	88,87± 40,48	109,82± 23,47	92,53± 22,77	48,72± 17,41	53,31± 7,43	97,49± 31,73	116,35± 42,02	180,64± 52,01	28,05± 7,39
Tylenchidae	87,36± 19,53	56,44± 12,84	29,20± 27,01	176,60± 41,09	197,00± 34,56	15,61± 5,67	16,00± 3,76	27,80± 7,08	55,16± 12,91	1,54± 1,54
Tylenchorhynchus	61,28± 20,23	0	7,35± 7,35	0	0	0	0	0	0	0

Table S4.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Tylenchus	9,70± 5,90	5,12± 2,51	28,23± 7,70	24,87± 8,17	50,41± 17,14	35,58± 16,95	19,13± 6,85	17,45± 6,15	5,09± 3,69	119,49± 23,49
Xiphinema	0	0	0	0	1,29± 1,29	1,06± 1,06	0	2,67± 1,69	0	11,84± 3,27
Omnivores										
Actinolaimidae	0	0	0	0	6,78± 6,78	0	0	1,26± 1,26	0	3,61± 2,29
Aporcelaimellus	0	0	0	0	0	10,45± 7,29	1,28± 1,28	0	0	0
Aporcelaimidae	23,11± 8,09	13,30± 5,67	10,80± 6,30	19,90± 10,26	5,79± 4,44	1,26± 1,26	0	0	1,62± 1,62	2,73± 1,88
Aporcelaimus	0	0	0	0	0	7,65± 7,65	0	0	0	0
Dorylaimidae	9,84± 9,84	0	3,04± 1,96	2,76± 2,76	0	0	0	0	0	0
Eudorylaimus	4,76± 4,76	18,31± 7,26	22,11± 14,03	5,61± 4,15	1,21± 1,21	7,57± 2,83	5,44± 2,78	8,31± 6,95	2,68± 2,68	4,20± 4,20
Kochinema	0	0	0	0	0	0	0	0	0	3,01± 1,94
Mesodorylaimus	14,99± 7,60	18,29± 8,60	36,22± 21,70	2,88± 2,88	12,35± 4,69	13,39± 5,75	13,75± 5,29	19,83± 7,93	0	19,35± 6,13
Prodorylaimus	0	0	0	0	1,29± 1,29	2,55± 1,64	0,85± 0,85	0	1,62± 1,62	0
Thornenematidae	3,52± 2,24	13,29± 6,45	34,47± 14,00	5,77± 5,77	6,80± 4,57	9,40± 3,30	5,58± 2,81	3,28± 1,61	0	3,39± 2,16
Thornia	0	0	0	0	0	1,48± 1,48	0	3,43± 2,78	0	0
Predators										

Table S4.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Anatonchidae	0	0	0	0	0	0	0	0	0	1,28± 1,28
Clarkus	0	0	3,42± 3,42	0	0	0	0	0	0	0
Coomansus	0	0	2,54± 2,54	0	3,53± 2,36	0	0	0	0	0
Discolaimidae	2,08± 2,08	0	0	2,80± 2,80	0	0	0,95± 0,95	1,41± 1,41	0	0,88± 0,88
Discolaimoides	0	0	0	0	0	1,26± 1,26	0	0	0	0
Mononchidae	0	0	0	0	0	1,48± 1,48	1,12± 1,12	0,60± 0,60	0	8,70± 4,68
Mononchus	0	0	0	0	0	0	0	4,05± 2,73	0	0
Mylonchulus	0	2,95± 3,54	0	2,80± 2,80	0	0	2,13± 1,39	2,67± 1,69	0	0
Nordiidae	11,55± 6,12	2,10± 2,52	9,49± 5,17	2,76± 2,76	9,93± 4,14	5,77± 4,38	2,92± 1,32	5,35± 2,59	3,24± 2,08	13,30± 6,82
Nygolaimidae	12,07± 5,64	10,29± 5,03	10,01± 5,46	16,70± 7,79	6,11± 2,93	5,25± 3,76	8,21± 4,09	5,31± 2,64	0	7,59± 3,37
Oxydirus	0	0	0	0	0	0	0	1,22± 1,22	0	0
Qudsianematidae	7,56± 2,55	27,03± 4,16	14,44± 9,76	36,48± 8,05	28,80± 7,03	39,23± 17,07	23,71± 8,66	12,88± 4,58	4,62± 3,25	16,87± 3,97
Tripylidae	8,04± 2,67	14,21± 6,34	21,35± 12,51	0	16,64± 5,08	7,79± 4,84	9,88± 5,14	16,32± 6,21	0	13,03± 5,97

Table S4.1b: Average amount of nematodes extracted from 200 g of soil and the mean and standard error of the mean of the ten most prevalent occurring nematode taxa in three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede during the first sampling interval.

	First Sampling interval				
	VCA1	**VCA2**	**VCA3**	**VConv**	**NVV**
Average amount of nematodes per sampling site	1205	1145	1136	1916	936
1st most prevalent taxa	*Pratylenchus* 212,09± 51,85	*Rhabditis* 207,68± 43,90	*Pratylenchus* 29,50± 30,94	*Cephalobus* 267,12± 28,21	Tylenchidae 197,00± 34,56
2nd most prevalent taxa	*Cephalobus* 140,43± 25,36	*Cephalobus* 115,70± 18,25	*Cephalobus* 124,24± 37,00	*Aphelenchus* 226,47± 51,68	Criconematidae 57,66± 27,81
3rd most prevalent taxa	*Aphelenchus* 94,75± 18,11	*Pratylenchus* 112,57± 16,14	*Aphelenchus* 117,89± 25,60	Tylenchidae 176,60± 41,09	*Tylenchus* 50,41± 17,14
4th most prevalent taxa	Tylenchidae 87,36± 19,53	*Aphelenchus* 106,53± 21,64	*Scutellonema* 109,82± 23,47	*Pratylenchus* 154,25± 44,83	*Scutellonema* 48,72± 17,41
5th most prevalent taxa	*Scutellonema* 75,67± 21,42	*Eucephalobus* 103,19± 32,06	*Plectus* 77,20± 29,89	*Eucephalobus* 130,83± 38,78	*Tylencholaimus* 34,54± 15,12
6th most prevalent taxa	*Tylenchorhynchus* 61,28± 20,23	*Scutellonema* 88,87± 40,48	*Mesodorylaimus* 36,22± 21,70	*Anaplectus* 102,92± 31,60	*Belondira* 33,35± 13,29
7th most prevalent taxa	*Plectus* 54,36± 18,20	*Anaplectus* 60,20± 20,40	Thornenematidae 34,47± 14,00	*Scutellonema* 92,53± 22,77	*Acrobeles* 32,98± 8,77
8th most prevalent taxa	*Eucephalobus* 51,84± 15,69	*Tylenchidae* 56,44± 12,84	*Eucephalobus* 32,38± 5,83	*Acrobeles* 83,40± 45,62	*Rotylenchulus* 32,80± 11,99
9th most prevalent taxa	*Anaplectus* 38,50± 12,18	*Rotylenchulus* 28,92± 13,06	*Panagrolaimus* 30,37± 19,28	*Aphelenchoides* 59,44± 25,64	*Aphelenchus* 31,59± 12,80
10th most prevalent taxa	*Prismatolaimus* 30,37± 6,98	Qudsianematidae 27,03± 4,16	Tylenchidae 29,20± 27,01	*Plectus* 53,33± 24,23	Qudsianematidae 28,80± 7,03

Table S4.1c: Average amount of nematodes extracted from 200 g of soil and the mean and standard error of the mean of the ten most prevalent occurring nematode taxa in three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede during the second sampling interval.

	Second Sampling interval				
	VCA1	**VCA2**	**VCA3**	**VConv**	**NVV**
Average amount of nematodes per sampling site	935	781	759	1070	877
1st most prevalent taxa	*Pratylenchus* 158,45± 29,77	*Aphelenchus* 101,80± 21,12	*Scutellonema* 116,35± 42,02	*Scutellonema* 180,64± 52,01	*Tylenchus* 119,49± 23,49
2nd most prevalent taxa	*Aphelenchus* 85,81± 21,39	*Scutellonema* 97,49± 31,73	*Aphelenchus* 111,27± 14,13	*Aphelenchus* 145,24± 27,10	*Filenchus* 111,83± 29,95
3rd most prevalent taxa	*Eucephalobus* 69,05± 50,88	*Pratylenchus* 91,01± 22,40	*Pratylenchus* 80,95± 8,47	*Rotylenchulus* 109,54± 59,58	*Helicotylenchus* 85,55± 21,20
4th most prevalent taxa	*Dolichodoridae* 56,99± 21,63	*Eucephalobus* 61,09± 17,51	*Cephalobus* 47,59± 12,76	*Cephalobus* 96,94± 14,36	*Belondira* 55,34± 22,58
5th most prevalent taxa	*Scutellonema* 53,31± 7,43	*Cephalobus* 43,05± 18,88	*Plectus* 40,54± 8,44	*Pratylenchus* 94,31± 21,86	*Tylencholaimus* 42,15± 23,26
6th most prevalent taxa	*Filenchus* 52,23± 13,35	*Filenchus* 40,31± 11,11	*Eucephalobus* 37,05± 11,06	*Eucephalobus* 75,28± 10,69	*Aphelenchoides* 37,09± 7,44
7th most prevalent taxa	Qudsianematidae 39,23± 17,07	*Helicotylenchus* 31,44± 12,31	Tylenchidae 27,80± 7,08	*Filenchus* 58,78± 11,23	*Acrobeles* 36,18± 14,71
8th most prevalent taxa	*Cephalobus* 39,06± 10,70	*Rhabditis* 28,91± 10,20	*Rotylenchulus* 21,25± 8,07	Tylenchidae 55,16± 12,91	*Scutellonema* 28,05± 7,39
9th most prevalent taxa	*Tylenchus* 35,58± 16,95	Qudsianematidae 23,71± 8,66	*Mesodorylaimus* 19,83± 7,93	*Helicotylenchus* 51,31± 19,24	Dolichodoridae 27,86± 7,53
10th most prevalent taxa	*Plectus* 34,62± 12,38	*Rotylenchulus* 22,48± 10,89	*Tylenchus* 17,45± 6,15	*Plectus* 32,83± 11,55	*Aphelenchus* 20,62± 7,67

Table S4.2: Mean ± standard error of the mean for the abiotic parameters of three conservation agriculture farmlands (VCA1-3), one conventional agriculture farmland (VConv), and a natural veld area/reference site (NVV) in Vrede during two sampling intervals.

	First Sampling Interval					Second Sampling Interval				
Abiotic parameter	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
% Sand	21,03 ± 3,20	18,26 ± 1,04	23,82 ± 1,02	27,30 ± 1,63	21,38 ± 1,22	18,14 ± 1,02	17,22 ± 1,31	24,05 ± 2,14	27,29 ± 1,79	19,61 ± 1,40
% Silt	23,69 ± 1,34	27,23 ± 0,86	23,16 ± 1,57	23,83 ± 1,01	30,16 ± 1,10	24,69 ± 0,44	26,57 ± 0,57	22,05 ± 0,52	28,42 ± 6,18	28,63 ± 1,28
% Clay	55,28 ± 2,46	54,52 ± 1,45	53,03 ± 2,24	48,87 ± 2,04	48,46 ± 1,22	57,16 ± 0,79	56,21 ± 1,37	53,91 ± 1,95	44,29 ± 7,52	51,76 ± 1,87
pH	6,28 ± 0,07	6,22 ± 0,07	6,30 ± 0,04	6,48 ± 0,10	6,18 ± 0,04	6,37 ± 0,04	6,22 ± 0,05	6,43 ± 0,03	6,12 ± 0,05	6,38 ± 0,05
EC	121,67 ± 14,93	66,67 ± 3,33	96,67 ± 15,63	105,00 ± 5,63	100,00 ± 6,83	145,00 ± 7,64	153,33 ± 9,19	143,33 ± 9,55	153,33 ± 8,43	168,33 ± 10,78
Na	42,46 ± 3,41	28,57 ± 0,92	27,54 ± 1,49	30,57 ± 2,81	41,87 ± 2,69	39,33 ± 2,20	31,33 ± 2,09	31,17 ± 1,94	32,00 ± 3,76	37,17 ± 4,71
K	161,33 ± 18,88	228,67 ± 7,93	192,17 ± 10,26	295,17 ± 21,21	264,17 ± 39,07	134,00 ± 11,77	173,67 ± 10,83	140,67 ± 16,17	184,83 ± 26,77	161,67 ± 23,33
Ca	2818,17 ± 129,01	2372,00 ± 56,42	2270,83 ± 93,40	1926,17 ± 201,39	2951,67 ± 41,23	2799,00 ± 200,67	2349,17 ± 87,52	2274,17 ± 115,74	1777,83 ± 146,22	2734,83 ± 105,05
Mg	2893,17 ± 152,37	2569,17 ± 142,75	2351,67 ± 71,64	1936,67 ± 160,92	2685,83 ± 99,22	2779,33 ± 153,28	2508,83 ± 184,88	2336,00 ± 151,00	1794,67 ± 230,81	2458,00 ± 134,43
CEC	38,40 ± 0,99	33,62 ± 1,15	31,23 ± 0,79	26,38 ± 1,00	37,63 ± 1,00	37,30 ± 1,63	32,87 ± 1,78	31,02 ± 1,48	24,20 ± 1,50	34,38 ± 1,52
S	19,51 ± 1,18	19,01 ± 0,27	19,05 ± 0,95	17,83 ± 1,34	16,72 ± 1,02	18,53 ± 0,93	18,07 ± 0,79	17,59 ± 0,80	16,77 ± 1,56	16,92 ± 0,56
P	28,63 ± 7,63	25,03 ± 4,89	22,11 ± 6,88	28,67 ± 5,00	13,52 ± 1,91	36,01 ± 7,91	11,94 ± 2,63	20,34 ± 5,78	30,95 ± 6,39	20,23 ± 2,41
B	2,03 ± 0,05	2,01 ± 0,03	1,90 ± 0,04	1,93 ± 0,03	2,27 ± 0,04	1,92 ± 0,02	1,94 ± 0,06	1,74 ± 0,05	1,62 ± 0,05	1,95 ± 0,03
Fe	144,48 ± 8,07	162,93 ± 7,17	142,74 ± 3,67	136,71 ± 5,56	143,09 ± 9,98	120,29 ± 1,77	139,08 ± 2,59	118,90 ± 4,25	109,54 ± 7,00	117,97 ± 6,94
Mn	102,25 ± 3,84	103,14 ± 2,53	112,39 ± 4,70	110,03 ± 4,62	81,79 ± 5,71	57,49 ± 2,59	52,11 ± 2,04	57,54 ± 5,27	51,43 ± 5,18	29,75 ± 1,82

Table S4.2: Continued

	First Sampling Interval					Secoond Sampling Interval				
Abiotic parameter	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**	**VCA1**	**VCA2**	**VCA3**	**VConv**	**NVV**
Cu	6,70 ± 0,29	6,79 ± 0,17	5,55 ± 0,22	5,81 ± 0,43	4,31 ± 0,40	5,80 ± 0,28	6,12 ± 0,37	4,35 ± 0,20	4,38 ± 0,39	3,57 ± 0,24
Zn	2,21 ± 0,29	2,45 ± 0,28	2,16 ± 0,03	7,32 ± 4,21	1,43 ± 0,14	1,60 ± 0,09	1,94 ± 0,14	1,45 ± 0,06	6,77 ± 4,23	0,97 ± 0,20
Al	1013,34 ± 52,21	1017,28 ± 17,12	973,61 ± 21,66	960,57 ± 31,16	809,66 ± 47,44	867,90 ± 22,70	837,74 ± 23,87	812,98 ± 25,82	758,76 ± 28,91	683,61 ± 37,56
Mo	0,10 ± 0,03	0,10 ± 0,02	0,15 ± 0,02	0,09 ± 0,02	0,13 ± 0,03	0,11 ± 0,03	0,11 ± 0,03	0,09 ± 0, 01	0,11 ± 0,02	0,07 ± 0,03
Co	2,13 ± 0,12	2,45 ± 0,13	2,51 ± 0,15	2,35 ± 0,20	1,50 ± 0,12	1,39 ± 0,07	1,58 ± 0,09	1,51 ± 0,15	1,15 ± 0,15	0,70 ± 0,06
Ni	13,53 ± 0,66	21,59 ± 1,44	12,20 ± 0,19	9,75 ± 0,64	10,35 ± 1,04	8,18 ± 0,25	14,29 ± 1,16	7,14 ± 0,32	6,25 ± 0,56	6,37 ± 0,47
Inorganic N	20,91 ± 4,94	14,24 ± 0,80	18,82 ± 1,83	25,58 ± 1,94	14,02 ± 1,42	26,31 ± 1,79	26,59 ± 1,21	27,46 ± 1,33	35,96 ± 2,93	23,08 ± 2,11

Table S5.1a: Mean ± standard error of the mean for the abundance of nematode families and genera (per 200 g of soil) found in three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv) and one natural veld area/reference site (NVR) in Reitz during two sampling intervals. Family and genera classification under respective trophic groups are based on the Nemaplex classification.

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Bacterivores										
Acrobeles	87,83± 26,19	72,57± 25,33	198,65± 62,26	111,20± 38,73	66,59± 23,66	165,12± 54,19	6,97± 22,86	99,16± 38,32	142,56± 44,54	1,60± 2,87
Acrobeloides	1,85± 1,85	0	0	0	0	0	4,73± 4,73	0	0	0
Alaimidae	0	0	0`	8,37± 8,37	9,48± 7,87	2,73± 2,73	0	0	0	0
Alaimus	0	0	8,35± 8,35	7,76± 7,76	1,92± 1,92	0	0	0	0	0
Anaplectus	0	3,22± 3,22	18,47± 9,50	8,95± 8,95	0	5,86± 3,77	1,94± 5,13	23,62± 14,54	0	2,43± 2,43
Cephalobidae	0	0	0	0	0	5,41± 3,45	12,80± 9,38	29,65± 15,81	19,76± 12,59	1,74± 1,74
Cephalobus	15,15± 18,82	34,89± 11,84	141,26± 72,91	69,37± 16,26	53,28± 31,77	175,56± 37,17	99,93± 23,63	56,86± 22,37	59,39± 18,91	58,71± 1,16
Cervidellus	16,25± 12,14	0	8,13± 8,13	3,89± 2,14	0	14,59± 8,45	0	13,14± 8,39	2,23± 1,82	15,37± 6,45
Chiloplacus	1,85± 1,85	8,98± 7,51	1,87± 6,96	16,28± 11,13	1,44± 1,44	0	0	0	0	0
Cruznema	0	0	0	0	0	0	0	0	0	2,43± 2,43
Diplogastridae	0	0	0	0	0	0	0	0	0	0
Eucephalobus	44,88± 12,86	58,95± 27,80	211,19± 24,45	46,82± 22,47	4,33± 4,33	19,30± 7,30	31,35± 8,15	35,76± 17,22	55,79± 2,63	11,57± 6,93

Table S5.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Mesorhabditis	3,96± 2,52	0	6,66± 4,23	2,98± 2,98	1,71± 1,71	3,36± 3,36	0	0	0	11,67± 8,99
Monhysteridae	0	1,92± 1,92	0	1,49± 1,49	3,84± 3,84	0	4,73± 4,73	3,97± 3,97	0	5,23± 5,23
Panagrolaimus	1,45± 7,00	16,60± 5,79	37,25± 15,57	4,24± 3,66	0	11,59± 8,74	14,86± 4,94	38,65± 27,85	0	7,48± 5,15
Plectus	1,64± 1,64	36,51± 32,16	57,46± 26,15	9,47± 8,86	45,47± 33,22	0	0	19,60± 12,73	0	2,53± 2,53
Prismatolaimus	1,64± 1,64	61,88± 25,42	0	0	14,94± 5,65	3,16± 3,16	72,67± 24,93	17,50± 17,50	0	29,75± 8,29
Rhabditidae	0	0	0	0	26,78± 26,78	3,36± 3,36	5,15± 5,15	5,92± 5,92	0	0
Rhabditis	1,84± 1,84	0	0	6,34± 4,49	8,34± 8,34	0	0	0	0	0
Tylocephalus	1,44± 1,44	0	4,15± 4,15	1,49± 1,49	3,36± 2,16	0	0	5,92± 5,92	0	4,95± 3,13
Zeldia	43,49± 16,28	8,58± 5,46	4,15± 4,15	6,51± 3,59	4,89± 3,11	9,25± 15,86	17,35± 7,66	14,61± 9,52	22,17± 8,52	1,74± 1,74
Fungivores										
Aphelenchoides	11,14± 4,18	6,43± 6,43	61,47± 55,97	5,64± 3,70	5,54± 2,54	0	5,83± 3,68	0	49,87± 29,30	26,84± 7,55
Aphelenchus	239,78± 58,52	75,48± 14,45	182,86± 41,26	92,42± 24,24	75,86± 27,48	21,95± 4,67	82,28± 3,61	58,70± 13,42	71,86± 29,62	63,96± 17,30
Diphtherophora	11,61± 5,93	8,69± 4,70	12,85± 8,53	2,79± 2,79	23,62± 5,74	19,97± 5,53	2,95± 13,43	8,34± 5,29	16,92± 11,33	32,52±7 ,88
Dorylaimoides	0	0	0	0	0	0	0	0	0	0

Table S5.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Filenchus	12,93± 8,29	24,90± 14,86	0	29,55± 26,60	8,85± 8,85	0	0	0	0	55,54± 25,48
Leptonchidae	0	0	0	0	6,19± 4,26	0	0	0	0	2,43± 2,43
Nothotylenchus	5,52± 5,52	9,65± 9,65	7,20± 7,20	24,84± 18,95	12,50± 9,47	0	0	0	0	0
Tylencholaimellus	0	0	0	0	0	0	0	0	0	0
Tylencholaimidae	0	0	0	0	0	0	0	5,83± 5,83	0	0
Tylencholaimus	0	0	0	0	15,70± 8,52	3,16± 3,16	0	5,83± 5,83	0	6,15± 3,98
Neotylenchidae	1,84± 1,84	0	0	1,43± 1,43	21,72± 11,94	0	0	0	0	6,58± 3,94
Herbivores										
Axonchium	0	0	0	0	0	0	0	3,97± 3,97	0	2,43± 2,43
Belondira	0	0	0	1,49± 1,49	15,15± 7,24	0	0	0	0	0
Belondiridae	1,85± 1,85	0	0	1,23± 1,23	4,32± 4,32	0	0	0	3,11± 3,11	0
Coslenchus	0	0	0	0	0	0	0	0	0	3,63± 3,63
Boleodorus	0	0	3,62± 3,62	0	0	0	0	0	0	0
Criconematidae	1,64± 1,64	0	0	2,39± 1,83	11,67± 31,25	0	0	0	0	0

Table S5.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Dolichodoridae	0	0	0	0	0	3,36± 3,36	11,88± 11,88	19,74± 9,32	0	39,52± 13,22
Ecphyadophoroides	3,38± 2,14	0	0	0	6,26± 2,87	0	7,30± 4,64	0	0	21,74± 9,45
Helicotylenchus	0	0	0	0	0	9,36± 6,26	99,18± 38,32	129,68± 45,24	27,78± 13,76	61,68± 23,24
Hemicycliophora	5,77± 5,77	0	0	0	0	18,32± 9,50	0	0	0	0
Hoplolaimidae	0	0	0	0	0	0	7,30± 4,64	5,83± 5,83	17,38± 17,38	2,29± 2,29
Longidorella	0	0	0	2,46± 2,46	0	0	0	0	3,11± 3,11	0
Longidoridae	0	0	0	1,43± 1,43	0	2,73± 2,73	0	9,80± 6,36	17,46± 13,16	1,74± 1,74
Longidorus	0	0	0	0	0	0	0	0	0	6,81± 2,82
Paratylenchus	0	0	0	0	0	0	0	0	4,56± 4,56	2,29± 2,29
Pratylenchus	142,93± 18,31	114,63± 25,84	137,43± 35,50	132,80± 24,31	61,67± 33,87	128,32± 45,70	313,19± 54,79	144,91± 58,15	16,70± 65,49	15,92± 3,57
Psilenchus	0	0	0	0	0	0	0	0	0	3,56± 2,26
Rotylenchulus	3,95± 2,58	155,50± 56,99	122,31± 34,48	156,58± 78,67	23,94± 1,66	134,75± 11,43	475,63± 153,87	738,25± 116,17	216,63± 515,83	53,62± 2,11
Scutellonema	22,35± 7,68	424,22± 126,11	1552,42± 32,54	234,39± 89,49	259,28± 44,42	57,82± 18,63	346,47± 152,59	1648,97± 537,33	437,22± 153,78	181,50± 37,38
Trichodorus	168,82± 15,76	21,24± 1,32	16,26± 16,26	11,56± 11,56	16,60± 5,79	383,47± 55,65	176,14± 47,37	18,59± 14,37	3,39± 11,33	29,41± 1,33

Table S5.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Tylenchidae	47,62± 18,58	19,67± 1,75	2,89± 9,62	21,91± 9,52	128,62± 48,97	52,47± 21,16	29,39± 11,68	22,93± 11,43	44,78± 24,56	135,38± 37,77
Tylenchorhynchus	0	7,84± 7,84	14,24± 14,24	0	0	0	0	0	0	0
Tylenchus	3,48± 3,48	11,58± 6,52	38,14± 23,85	7,30± 3,72	28,64± 14,66	1,97± 5,23	3,96± 3,96	5,92± 5,92	1,87± 1,87	97,71± 34,86
Xiphinema	0	0	0	5,25± 3,79	9,47± 4,60	0	0	0	0	8,13± 4,41
Omnivores										
Aporcelaimellus	0	0	0	0	0	22,38± 13,65	1,72± 1,72	0	0	3,99± 3,99
Aporcelaimidae	0	7,89± 5,72	0	28,14± 9,72	23,74± 4,73	8,52± 6,25	7,92± 7,92	5,92± 5,92	0	6,24± 2,83
Dorydorella	0	0	0	1,86± 1,86	0	0	0	0	0	0
Dorylaimidae	0	1,31± 1,31	0	0	6,63± 6,63	0	0	0	0	0
Eudorylaimus	0	4,53± 3,23	0	4,48± 4,48	4,84± 3,87	2,73± 2,73	4,73± 4,73	1,22± 6,56	0	0
Kochinema	0	0	0	5,28± 3,74	0	0	0	0	0	0
Mesodorylaimus	0	13,20± 7,54	0	8,69± 7,31	22,57± 15,37	0	0	0	0	0
Prodorylaimus	0	0	0	0	0	3,16± 3,16	42,28± 18,67	0	0	0
Thornenematidae	0	8,22± 4,34	0	4,28± 2,89	22,37± 1,53	0	0	5,83± 5,83	0	1,74± 1,74

Table S5.1a: Continued

	First Sampling interval					Second Sampling interval				
Trophic group/Family/Genus	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Predators										
Anatonchidae	0	0	0	0	1,92 ± 1,92	0	0	0	0	0
Coomansus	0	1,52 ± 1,52	0	0	1,92 ± 1,92	0	0	0	0	0
Discolaimidae	3,18 ± 2,26	2,61 ± 2,61	0	0	2,16 ± 2,16	0	0	0	0	0
Discolaimoides	0	0	0	0	2,17 ± 2,17	31,83 ± 11,63	6,74 ± 4,36	25,53 ± 13,30	4,45 ± 4,45	0
Discolaimus	0	0	0	0	0	0	0	0	0	6,24± 2,83
Mononchidae	0	0	0	0	0	3,16± 3,16	4,74± 3,16	0	0	5,50± 5,50
Mononchus	0	0	0	0	0	0	0	0	0	6,46± 2,94
Nordiidae	1,84± 1,84	7,97± 2,99	0	4,58± 2,17	11,25± 8,68	24,57± 18,78	5,67± 3,99	5,92± 5,92	17,79± 9,14	29,63± 7,65
Nygolaimidae	15,33± 9,64	12,79± 9,48	0	11,23± 3,19	1,11± 5,21	11,54± 3,78	6,12± 3,89	3,95± 3,95	0	2,51± 8,89
Oxydirus	0	4,29± 4,29	0	0	0	0	0	0	0	0
Qudsianematidae	17,73± 7,84	15,42± 11,12	8,13± 8,13	16,23± 7,45	28,92± 9,37	83,59± 18,50	9,14± 5,83	13,45± 6,79	24,83± 21,33	26,70± 6,19
Tripylidae	0	3,66± 2,37	0	11,94± 11,94	7,51± 3,18	0	0	0	4,45± 4,45	2,00± 2,00

Table S5.1b: Average amount of nematodes extracted from 200 g of soil and the mean and standard error of the mean of the ten most prevalent occurring nematode taxa in three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv), and a natural veld area/reference site (NVR) in Reitz during the first sampling interval.

	First Sampling interval				
	RCA1	**RCA2**	**RCA3**	**RConv**	**NVR**
Average amount of nematodes per sampling site	1099	1364	3144	1219	1302
1st most prevalent taxa	*Aphelenchus* 239,78± 58,52	*Scutellonema* 424,22± 126,11	*Scutellonema* 1552,42± 32,54	*Scutellonema* 234,39± 89,49	*Scutellonema* 259,28± 44,42
2nd most prevalent taxa	*Trichodorus* 168,82± 15,76	*Rotylenchulus* 155,50± 56,99	*Eucephalobus* 211,19± 24,45	*Rotylenchulus* 156,58± 78,67	Tylenchidae 128,62± 48,97
3rd most prevalent taxa	*Cephalobus* 15,15± 18,82	*Pratylenchus* 114,63± 25,84	*Acrobeles* 198,65± 62,26	*Pratylenchus* 132,80± 24,31	Criconematidae 11,67± 31,25
4th most prevalent taxa	*Pratylenchus* 142,93± 18,31	*Aphelenchus* 75,48± 14,45	*Aphelenchus* 182,86± 41,26	*Acrobeles* 111,20± 38,73	*Aphelenchus* 75,86± 27,48
5th most prevalent taxa	*Acrobeles* 87,83± 26,19	*Acrobeles* 72,57± 25,33	*Cephalobus* 141,26± 72,91	*Aphelenchus* 92,42± 24,24	*Acrobeles* 66,59± 23,66
6th most prevalent taxa	Tylenchidae 47,62± 18,58	*Prismatolaimus* 61,88± 25,42	*Pratylenchus* 137,43± 35,50	*Cephalobus* 69,37± 16,26	*Pratylenchus* 61,67± 33,87
7th most prevalent taxa	*Eucephalobus* 44,88± 12,86	*Eucephalobus* 58,95± 27,80	*Rotylenchulus* 122,31± 34,48	*Eucephalobus* 46,82± 22,47	*Cephalobus* 53,28± 31,77
8th most prevalent taxa	*Zeldia* 43,49± 16,28	*Plectus* 36,51± 32,16	*Aphelenchoides* 61,47± 55,97	*Filenchus* 29,55± 26,60	*Plectus* 45,47± 33,22
9th most prevalent taxa	*Scutellonema* 22,35± 7,68	*Cephalobus* 34,89± 11,84	*Plectus* 57,46± 26,15	Aporcelaimidae 28,14± 9,72	Qudsianematidae 28,92± 9,37
10th most prevalent taxa	Qudsianematidae 17,73± 7,84	*Filenchus* 24,90± 14,86	*Tylenchus* 38,14± 23,85	*Nothotylenchus* 24,84± 18,95	*Tylenchus* 28,64± 14,66

Table S5.1c: Average amount of nematodes extracted from 200 g of soil and the mean and standard error of the mean of the ten most prevalent occurring nematode taxa in three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv), and a natural veld area/reference site (NVR) in Reitz during the second sampling interval.

	Second Sampling interval				
	RCA1	**RCA2**	**RCA3**	**RConv**	**NVR**
Average amount of nematodes per sampling site	1753	2004	3257	3535	1350
1st most prevalent taxa	*Trichodorus* 383,47± 55,65	*Rotylenchulus* 475,63± 153,87	*Scutellonema* 1648,97± 537,33	*Rotylenchulus* 216,63± 515,83	*Scutellonema* 181,50± 37,38
2nd most prevalent taxa	*Aphelenchus* 21,95± 4,67	*Scutellonema* 346,47± 152,59	*Rotylenchulus* 738,25± 116,17	*Scutellonema* 437,22± 153,78	Tylenchidae 135,38± 37,77
3rd most prevalent taxa	*Cephalobus* 175,56± 37,17	*Pratylenchus* 313,19± 54,79	*Pratylenchus* 144,91± 58,15	*Helicotylenchus* 27,78± 13,76	*Acrobeles* 1,60± 2,87
4th most prevalent taxa	*Acrobeles* 165,12± 54,19	*Trichodorus* 176,14± 47,37	*Helicotylenchus* 129,68± 45,24	*Acrobeles* 142,56± 44,54	*Tylenchus* 97,71± 34,86
5th most prevalent taxa	*Rotylenchulus* 134,75± 11,43	*Cephalobus* 99,93± 23,63	*Acrobeles* 99,16± 38,32	*Pratylenchus* 16,70± 65,49	*Aphelenchus* 63,96± 17,30
6th most prevalent taxa	*Pratylenchus* 128,32± 45,70	*Helicotylenchus* 99,18± 38,32	*Aphelenchus* 58,70± 13,42	*Aphelenchus* 71,86± 29,62	Helicotylenchus 61,68± 23,24
7th most prevalent taxa	*Zeldia* 9,25± 15,86	*Aphelenchus* 82,28± 3,61	*Cephalobus* 56,86± 22,37	*Cephalobus* 59,39± 18,91	*Cephalobus* 58,71± 1,16
8th most prevalent taxa	Qudsianematidae 83,59± 18,50	*Prismatolaimus* 72,67± 24,93	*Panagrolaimus* 38,65± 27,85	*Eucephalobus* 55,79± 2,63	*Filenchus* 55,54± 25,48
9th most prevalent taxa	*Scutellonema* 57,82± 18,63	*Acrobeles* 6,97± 22,86	*Eucephalobus* 35,76± 17,22	*Aphelenchoides* 49,87± 29,30	*Rotylenchulus* 53,62± 2,11
10th most prevalent taxa	Tylenchidae 52,47± 21,16	*Prodorylaimus* 42,28± 18,67	Cephalobidae 29,65± 15,81	Tylenchidae 44,78± 24,56	Dolichodoridae 39,52± 13,22

Table S5.2: Mean ± standard error of the mean for the abiotic parameters of three conservation agriculture farmlands (RCA1-3), one conventional agriculture farmland (RConv) and a natural veld area/reference site (NVR) in Reitz during two sampling intervals.

	First Sampling Interval					Second Sampling Interval				
Abiotic parameter	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**	**RCA1**	**RCA2**	**RCA3**	**RConv**	**NVR**
Sand %	77,12 ± 0,91	60,21 ± 3,00	53,72 ± 1,91	62,09 ± 1,61	67,90 ± 2,58	78,21 ± 1,01	58,39 ± 2,71	54,22 ± 2,17	63,86 ± 1,92	69,52 ± 1,34
Silt %	15,03 ± 0,47	27,15 ± 0,75	22,13 ± 0,68	20,43 ± 0,90	21,72 ± 1,70	14,29 ± 0,39	26,83 ± 1,11	22,12 ± 0,60	18,96 ± 0,85	20,91 ± 0,91
Clay %	7,85 ± 0,50	12,64 ± 2,67	24,16 ± 1,29	17,48 ± 0,83	10,39 ± 3,01	7,50 ± 0,67	14,78 ± 1,82	23,66 ± 1,63	17,18 ± 1,09	9,58 ± 0,52
pH	5,65 ± 0,14	5,72 ± 0,06	5,70 ± 0,20	4,92 ± 0,07	5,40 ± 0,03	5,60 ± 0,10	5,80 ± 0,28	5,62 ± 0,10	4,85 ± 0,08	5,57 ± 0,02
EC	40,00 ± 2,58	86,67 ± 5,58	110,00 ± 28,87	100,00 ± 2,58	35,00 ± 4,28	58,33 ± 1,67	120,00 ± 30,22	86,67 ± 6,67	110,00 ± 9,31	50,00 ± 3,65
Na	7,48 ± 0,52	23,17 ± 9,15	9,64 ± 1,16	9,52 ± 0,76	12,88 ± 1,25	6,95 ± 0,21	23,57 ± 9,45	8,76 ± 0,44	9,07 ± 0,52	12,44 ± 1,42
K	75,33 ± 4,86	61,50 ± 4,19	192,00 ± 25,71	255,83 ± 26,29	86,00 ± 8,75	63,33 ± 4,59	59,83 ± 6,15	221,83 ± 24,47	194,50 ± 7,94	89,17 ± 12,53
Ca	226,83 ± 28,88	617,50 ± 56,58	625,50 ± 110,26	305,83 ± 41,26	361,50 ± 31,95	186,83 ± 10,67	603,17 ± 30,81	622,33 ± 63,24	267,83 ± 25,99	356,33 ± 27,89
Mg	79,83 ± 19,41	120,33 ± 12,96	97,00 ± 15,63	97,83 ± 13,39	126,17 ± 14,93	54,83 ± 8,77	115,67 ± 9,57	97,67 ± 10,02	85,00 ± 8,62	132,17 ± 17,47
CEC	2,02 ± 0,32	4,33 ± 0,41	4,47 ± 0,73	3,03 ± 0,36	3,12 ± 0,28	1,57 ± 0,13	4,23 ± 0,22	4,50 ± 0,45	2,57 ± 0,21	3,17 ± 0,29
S	9,29 ± 0,55	15,38 ± 1,96	14,40 ± 1,19	12,00 ± 0,57	9,61 ± 0,25	9,87 ± 0,61	15,95 ± 2,40	15,57 ± 0,82	14,14 ± 0,97	7,97 ± 0,53
P	86,68 ± 10,28	72,82 ± 22,58	51,44 ± 23,62	19,49 ± 5,34	13,17 ± 3,86	86,94 ± 7,81	47,98 ± 14,26	25,78 ± 6,05	26,83 ± 9,87	25,19 ± 5,61
B	1,28 ± 0,02	2,01 ± 0,09	1,41 ± 0,06	1,62 ± 0,07	1,79 ± 0,04	1,24 ± 0,04	1,76 ± 0,07	1,42 ± 0,04	1,66 ± 0,05	1,60 ± 0,05
Fe	111,24 ± 7,72	309,20 ± 14,89	62,02 ± 6,39	158,85 ± 8,54	299,86 ± 22,29	108,60 ± 10,49	263,64 ± 23,36	55,08 ± 1,38	144,15 ± 4,28	249,08 ± 17,45

Table S5.2: Continued

	First Sampling Interval					Second Sampling Interval				
Abiotic parameter	VCA1	VCA2	VCA3	VConv	NVV	VCA1	VCA2	VCA3	VConv	NVV
Mn	66,78 ± 6,21	39,33 ± 4,43	137,61 ± 12,52	144,58 ± 9,19	30,35 ± 3, 48	45,32 ± 6,03	43,65 ± 2,81	108,29 ± 2,21	132,03 ± 13,23	22,01 ± 2,56
Cu	1,62 ± 0,10	1,91 ± 0,12	4,23 ± 0,53	3,20 ± 0,16	1,39 ± 0,12	1,36 ± 0,12	1,81 ± 0,12	4,45 ± 0,35	3,06 ± 0,13	1,22 ± 0,11
Zn	5,32 ± 0,65	4,85 ± 0,46	5,71 ± 1,66	3,36 ± 0,18	1,15 ± 0,10	4,23 ± 0,32	4,71 ± 0,27	3,59 ± 0,15	3,56 ± 0,22	1,17 ± 0,14
Al	510,48 ± 22,69	496,33 ± 58,27	771,70 ± 53,96	842,84 ± 27,26	445,74 ± 15,18	466,69 ± 20,81	424,31 ± 55,28	795,51 ± 27,00	798,64 ± 32,29	390,35 ± 25,08
Mo	0,06 ± 0,02	0,08 ± 0,02	0,11 ± 0,02	0,09 ± 0,04	0,09 ± 0,02	0,10 ± 0,02	0,09 ± 0,02	0,03 ± 0,01	0,10 ± 0,02	0,08 ± 0,03
Co	0,60 ± 0,07	0,30 ± 0,03	1,57 ± 0,15	0,95 ± 0,09	0,29 ± 0,04	0,38 ± 0,06	0,26 ± 0,01	1,31 ± 0,08	0,96 ± 0,13	0,18 ± 0,04
Ni	0,59 ± 0,16	0,60 ± 0,03	0,54 ± 0,05	1,06 ± 0,09	0,71 ± 0,08	0,31 ± 0,05	0,53 ± 0,03	0,63 ± 0,07	1,19 ± 0,13	0,69 ± 0,10
Inorganic N	12,11 ± 0,41	15,17 ± 0,57	23,21 ± 3,64	34,49 ± 2,42	13,95 ± 0,23	23,33 ± 1,80	27,18 ± 1,24	29,69 ± 0,61	41,93 ± 3,79	29,67 ± 2,82